LIMNOLOGY AND OCEANOGRAPHY

January, 1958

VOLUME III

NUMBER 1

The Effects of Winds on Water Levels on the New England Coast[1]

ARTHUR R. MILLER

Woods Hole Oceanographic Institution, Woods Hole, Mass.

ABSTRACT

Tidal records from Nantucket Sound, Cape Cod; Montauk, Long Island; and Portland, Maine, have been studied along with wind and barometric observations from each location. Results of this study show that departures from mean sea level due to wind may be resolved into two components. The first is a component which refers to symmetrical sinusoidal variation of sea level departure as a function of wind direction and applies to the outer coast and to the general level of Nantucket Sound in particular.

The second is an asymmetrical component which refers the responses of sea level at each observing station to local wind force, direction, fetch length, and local topography. Under different circumstances these components vary in relative importance in determining the local departure from mean sea level. In addition to wind direction and velocity, duration and size of wind field may determine whether one component is more important than the other.

The varying importance of each component probably governs the time-lag between peak winds and maximum sea level departures which will vary from several hours to as much as twenty hours.

INTRODUCTION

The coast of New England is subjected from time to time to extraordinarily high water, the scale of which is usually related to the intensity of storms. Most spectacular are the inundations that sometimes occur during hurricanes. The extra elevation of sea level over the normal tide during a hurricane may amount to as much as ten to fifteen feet (Redfield and Miller 1957). Elevations of this scale are uncommon and hence adequate data are rare. Related phenomena on a smaller scale but of greater frequency should provide data useful for a better understanding of unusually high waters. It is the purpose of this paper to explore the less spectacular but more frequent departures from mean sea level accompanying ordinary winter storms.

Where departures from normal water levels have been greater than the normal tide, common practice has been to identify these departures as storm surges. The term *surges* in this paper will refer to relatively short periods of excessive height occurring during passage of a storm. Apart from surging of water levels, another sea-level phenomenon accompanying a storm but of much longer duration is that of set-up (Miller 1957). The term *set-up* has been used to describe the total seaward slope of water surface from a shore line which is established and maintained by relatively steady winds.

These phenomena are difficult to separate analytically, one difficulty being the lack of firm distinction between definitions of surge

[1] Contribution #900 from the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. This study was initiated with the aid of the Office of Naval Research, U. S. Navy. Further assistance was made available through the National Hurricane Research Project, U. S. Weather Bureau. The help of the U. S. Coast and Geodetic Survey and the U. S. Weather Bureau in providing data is gratefully acknowledged.

and set-up. For this purpose the term set-up may be considered as slope of water surface developing *in situ* and surge as slope of water surface developing in *direction of propagation*. Since tidal observations are usually limited to the shoreline, measurements of slope are likewise limited. Consequently, with respect to a tide staff, the essential difference between the two phenomena is that surge is a rapid rise in water level with a duration of several hours or less, while set-up appears to be a relatively slow rise or fall of water level with durations of hours or days, depending on the persistence of winds and direction of winds.

This paper attempts to describe the amount of set-up observed to accompany winds of various strengths and directions at several different positions on the New England coast. Observations from six tide gauging stations were used (Fig. 1). Four of these stations were situated about Nantucket Sound, the other two at Portland, Maine, and Montauk, Long Island. Montauk is more or less exposed to the open sea from the south, Portland from the east, while Nantucket Sound is exposed from the east, south, or west. In addition to the different exposures of the three regions, the tidal characteristics vary greatly both in time of high water and in tidal range. For instance, Woods Hole in Nantucket Sound has a mean range of less than two feet while Portland has a range greater than nine feet.

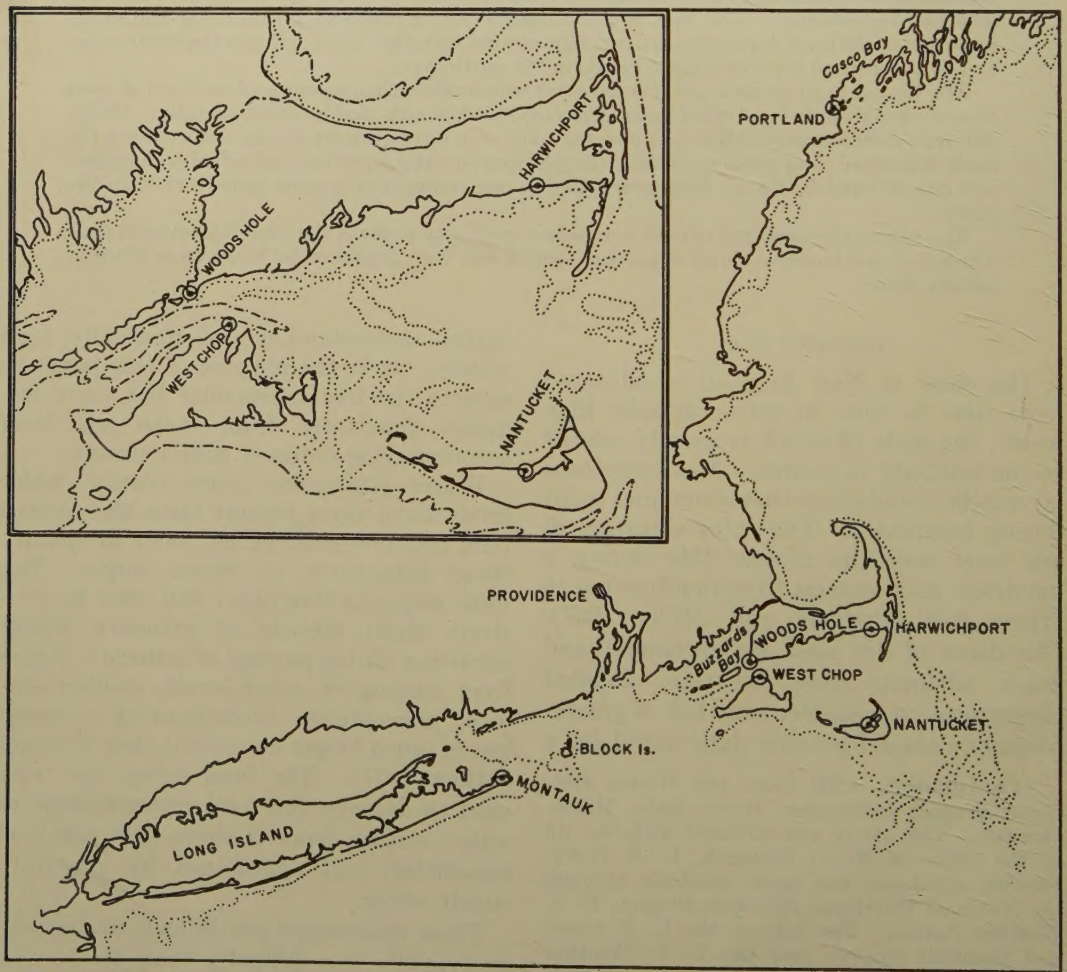

FIG. 1. Locations of tide gauges in New England and on Long Island. Insert is enlarged view showing Nantucket Sound area.

Within Nantucket Sound itself there is a difference of more than three hours between time of high water from one end to the other (Redfield 1953). The dissimilarity of the stations serves to emphasize those features within the analyzed data which are common to all six stations.

The tidal observations have been treated so that non-periodic effects are separated from the predictable periodic motions. The predicted tide if subtracted from the observations will give as a remainder the effects of non-periodic variation in barometric pressure, tangential stress of the wind, or other forces whatever they may be. These variations in sea level are referred to collectively as meteorological effect. Further refinement is obtained if the non-predictable periodic motion is eliminated by some means. The residue is called *non-tidal sea level*. Variations of non-tidal sea level are then assumed to be made of two parts: (1) *barometric effect*, and (2) set-up, attributed to the coastal effects of tangential wind stress on the sea surface.

The method for obtaining non-tidal sea level is the use of numerical filters to eliminate as much of the tidal "noise" from the data as possible, following the recommendations of Groves (1955). The advantage of the filtering system or weighted average over the subtraction of the predicted tide from observations is in this reduction of "noise" in the data and also, for the particular objectives of this study, the minimizing of short-duration oscillatory phenomena, such as long waves or surges. In fact, since the tidal curves about Nantucket Sound were not predictable in detail, the method of numerical filtering was the only suitable means to eliminate the tide. The specific filter used was that described by Doodson and Warburg (1941) to determine mean sea level for any hour (B 39 filter in Groves' tabulation).

NON-TIDAL SEA LEVEL

Nantucket Sound is a triangular area adjoining the southern shore of Cape Cod and separating the two islands, Martha's Vineyard and Nantucket, from the mainland (see insert in Fig. 1). The apex of this triangle which averages about 30 feet in depth funnels into a deeper channel-like area with average depth of 70 feet, called Vineyard Sound, which in turn is open to the ocean. The opening of the Sound to the east is partially closed to the ocean by land barriers extending from the Cape and Nantucket Island. Between the two islands there is an outlet to the ocean to the south, of lesser importance because of its limited depths.

Two of the gauging stations were in the eastern part of the Sound. To the north was the Harwichport gauge, and due south of Harwichport the Nantucket gauge. At the western end the two stations Woods Hole and West Chop were closer together. In spite of their closeness their tidal situations are different since Woods Hole is influenced in some degree by the passage entering into Buzzards Bay.

The tidal observations used were hourly heights obtained from October, 1953, to March, 1954. Figure 2 shows the results of numerical filtering of the data from Nantucket Sound, Montauk, and Portland for three representative storms. Each trace is referred to the monthly mean sea level of each station.

The concurrent non-tidal sea levels change in a very similar way at all of the six tide gauges. However, there were significant departures from this parallelism. For instance, during the northeast storm of November 7, the Montauk sea level rose to a height more than twice the height at Portland. There were periods when concurrent sea levels at Woods Hole or Nantucket differed appreciably from those at other stations. Frequently, there were times when no station differed from any other by more than a tenth of a foot. Thus, parallelism of non-tidal sea levels about New England is a loose, but real, generalization.

BAROMETRIC PRESSURE

In the passage of storms over the New England area there is usually a west to east motion. This motion is reflected in the barometric records. The relation is such that at times there will be parallelism in the pressure records among the stations similar

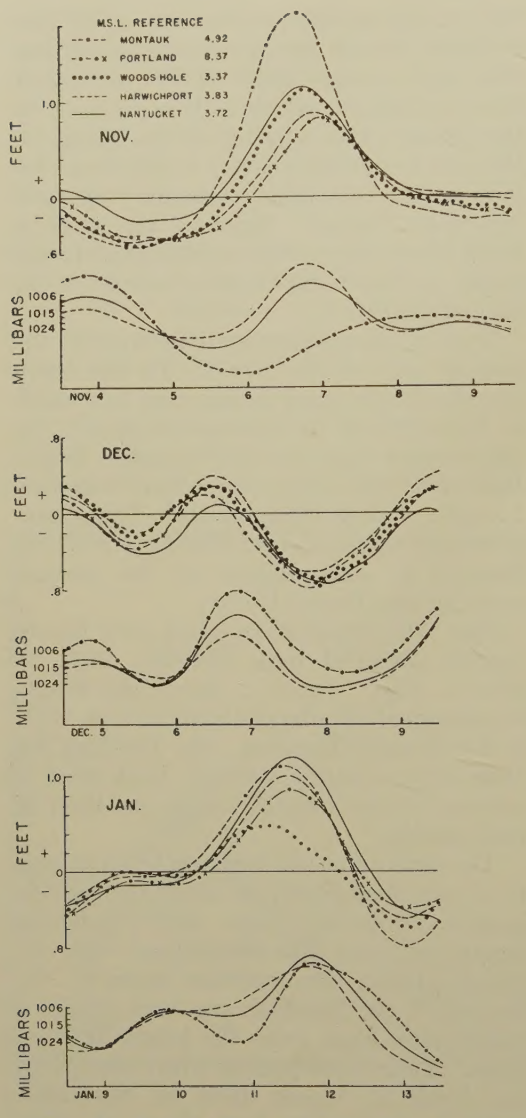

FIG. 2. Non-tidal sea levels during three storms with concurrent barometric pressures. The common datum for five stations is monthly mean sea level. Barometric pressures are shown for three general areas: Montauk - - - - -, Nantucket ————, Portland ·-·-·-·-·.

to the sea level phenomena, or just as frequently the interrelations may be quite dissimilar. Whether the records are similar or not will depend on the size, speed, and geographical distribution of the pressure disturbance.

Barometric pressures are shown in Figure 2 below the non-tidal sea levels for all three localities. The vertical scale for pressure is inverted so that a rising curve represents a drop in pressure. As sea level rises and falls, an inverse correspondence of rise and fall of barometric pressure usually accompanies the sea level variation. The parallelism between the different locations is less close in the case of barometric pressure than in that of non-tidal sea levels.

In most cases where correspondence between the two types of curves was clear the fluctuation of sea level preceded the fluctuations of pressure, the precedence varying from six to twenty-four hours. The precedence of a sea level event before an event in pressure has not gone unnoticed. In cases of tropical hurricanes and other severe storms an abnormal rise of water levels is sometimes taken as a sign of an approaching storm (Cline 1926). The effect has been noticed in the Gulf of Mexico and along the east coast. The comparisons of sea level and pressure for the New England coast show that this is a regular phenomena.

BAROMETRIC CORRECTION

When there is an unequal distribution of pressure over a sea surface the level of the sea will rise under low pressure and fall under high pressure in an amount commensurate with the difference between the densities of air and water. The theoretical amount of rise of fall is approximately 1 foot for 30 millibars of pressure if equilibrium is established. Where pressure changes with time the response of sea level will be nearly immediate provided the volume of water involved can flow unimpeded (Doodson and Warburg 1941). It is not certain whether the latter condition is met in a restricted shallow sea such as Nantucket Sound.

Observations of sea level when other forces are at a minimum should indicate if the theoretical value is valid. Non-tidal sea levels for two stations, Harwichport and West Chop, were selected at periods when wind force was less than 15 knots and compared with barometric readings for the same time. While there was considerable scatter of values of sea level against pressure, as would be expected in such rough determinations, these values clustered linearly, and

the results showed that theoretical relationship could be applied to the conditions at these stations.

With the pressure correction established empirically, the non-tidal sea levels were corrected for pressure. The sea levels resulting from the subtraction of the pressure fluctuations are shown in Figure 3 for the storms of December 7, September 2, and January 11. The subtraction of the pressure effect from the non-tidal sea levels altered the curves significantly—two feet in water level representing ranges of pressure up to 60 millibars as compared with ranges of non-tidal sea level up to three or four feet. The residue from this subtraction was assumed to be the variations of sea level resulting from wind set-up.

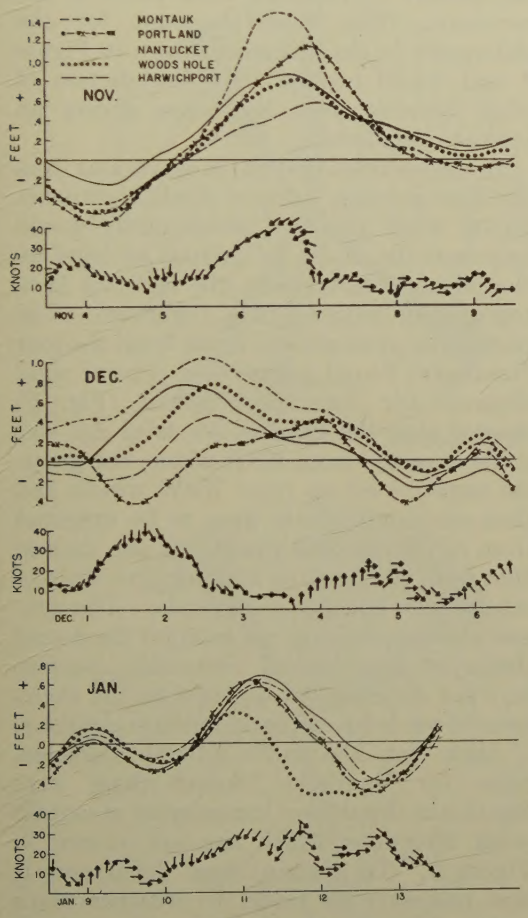

FIG. 3. Non-tidal sea levels corrected for effects of pressure. The common datum for five stations is monthly mean sea level. Observed winds at Nantucket are shown below each set of curves. Wind velocity is read on the knot scale opposite the arrow points.

WIND SET-UP

First attempts to relate the observed winds, examples of which are shown in Figure 3, to the observed set-up were wholly unsatisfactory. It was anticipated that the relation would be sinusoidal at each station, that is, rise and fall of sea level would be symmetrically responsive to some component of wind direction. At Atlantic City, sinusoidal symmetry was applicable in the correlation of set-up with geostrophic winds (Miller 1957). However, Atlantic City is part of a long, straight coastline. This is not the case with the New England coast where the coastline is broken by promontories, bays, and sounds.

Wind force acts on the sea surface as a frictional force on the skin layer; consequently, the velocity is maximal at the surface, decreasing with depth. If this motion is directed towards a coast, an elevation will result at the coastline which will continue to rise until the resulting slope of the surface is sufficient to balance the forces responsible for the motion. The development of elevation depends on transport of water to the coast; consequently, this phenomenon must follow the occurrence of winds giving rise to it. Also, an existing wind drift could conceivably resist casual wind shifts, since any change in the prevailing forces would have to be downwards before the general transport would be appreciably affected. The quality of steadiness of the wind, therefore, is important to the net amount of water being transported.

Non-tidal sea levels rise and fall aperiodically with time but fluctuate slowly enough to identify the time and height of each peak or trough. It was assumed that at each of these times of stationary sea level, a critical relation existed between the set-up and the wind force responsible for the fluctuation. Wind records from nearby weather stations were examined for the greatest velocity prior to the event of stationary sea level. The time, velocity, and direction were noted along with the time and height of set-up.

A time interval of less than 24 hours was considered to be a reasonable limit within which these comparisons were carried out. On a few occasions it was necessary to match sea level heights with early wind shifts where peak velocities were absent. The relation is not violated by the latter method since a peak velocity of some wind component is indicated by the shift of wind.

These selected heights of sea level were plotted on polar coordinates, where radial vectors corresponding to the direction and velocity of the wind were matched with the particular height. With the completion of the polar diagram, lines of equal height were drawn to fit the distribution of observations. During six months of observation full directional distribution of winds may be expected in New England, with some directions favored more than others. For the most part it was usually found that for a single wind direction there were a number of events to represent a full velocity spectrum up to 35 knots, and a linear relationship between set-up and wind was a reasonable description of the observations. With full directional distribution, very little interpolation was required for constructing the diagrams. The results of these diagrams were presumed to describe the sea level required to balance a given wind condition and to describe the characteristic conditions at the observing tide station.

The diagrams representing characteristic set-up for given winds are shown in Figure 4 for all 6 gauging stations. Lines of sea level are given in tenths of feet in terms of each local tide staff. The division between negative and positive set-up represents mean sea level at the station. This division was derived from the summation of all observations within the first 10-knot circle and also corresponds to the summation of the total monthly sea levels for the period of observation at each station. The diagrams are similar in general features but differ greatly from each other along critical wind directions. Nor do they show any features of symmetry except in the progressions in scale of set-up in adjoining compass directions. It is not clear from these diagrams how the scale of set-up is affected by local topography. They scarcely explain how a station, such as Harwichport, can exhibit a rise in sea level with the wind coming off the land.

THE SYMMETRICAL COMPONENT: THE GENERAL COASTAL COMPONENT

It is reasonable to assume that in a landlocked sea such as Nantucket Sound having free communication with the ocean a part of the set-up would result from a widespread change in the coastal non-tidal sea level induced by the general wind field over the off-lying ocean, and an additional part would result from the local winds and the topography surrounding each point of observation. This would account for the differences in the diagrams shown in Figure 4 and would explain the contradiction of wind blowing from the shore giving an increased sea level.

It was assumed that the observations from the four gauging stations about Nantucket Sound when averaged concurrently would represent the set-up as induced by wind on the offshore waters with the effect of local topography reduced to a minimum. The plot of the averaged sea levels from the four Nantucket Sound gauges over a polar wind diagram for Nantucket Sound (Fig. 5) showed that the greatest sea level rise was to be expected from ENE winds, the greatest negative set-up from WSW winds, and that minimum effects were to be expected from NNW and SSE winds. It also showed that both the positive and negative scale of set-up were equal in magnitude. The general characteristics of sea level for the Sound displayed symmetrical sinusoidal response to wind direction, in contrast to the skewness of sea level at the individual localities.

Aside from the above general characteristics for Nantucket Sound there were significant departures inconsistent in certain wind directions which are not shown in Figure 5. To explain these departures it was necessary to resort to weather maps rather than the locally observed winds at Nantucket. The inconsistencies fell into two groups: (1) light S–SW winds with time lags of 0–9 hours, and (2) strong NW–N winds with lags of 12–20 hours. In the

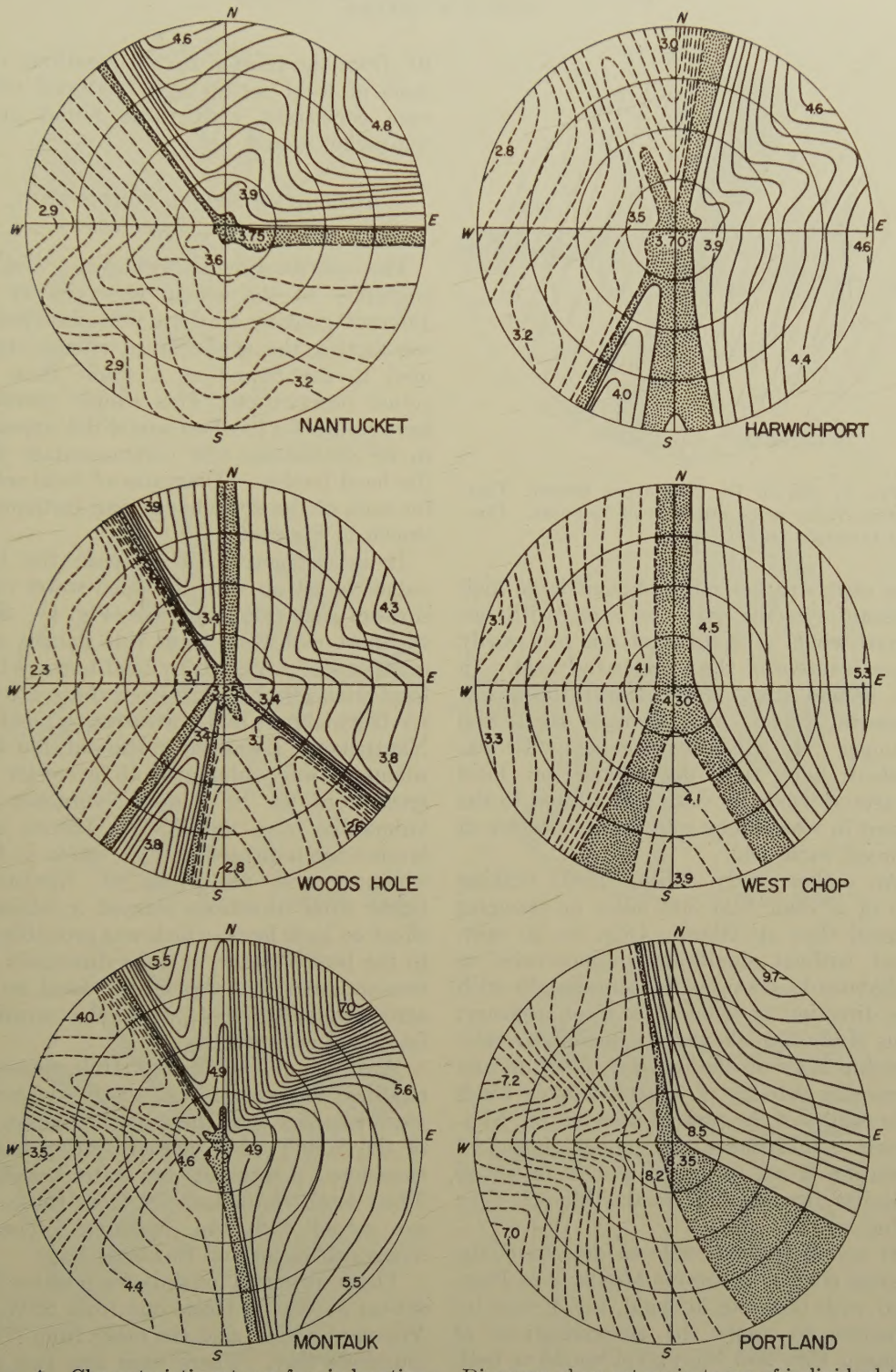

FIG. 4. Characteristic set-ups for six locations. Diagrams show set-up in terms of individual tide staffs for various wind vectors with speeds up to 40 knots. The concentric circles mark 10-knot intervals in wind speed. Shaded areas delineate neutral set-ups where winds have little or no effect on sea level. Dashed isopleths show negative set-ups and full isopleths positive set-ups, with intervals of 0.1 foot.

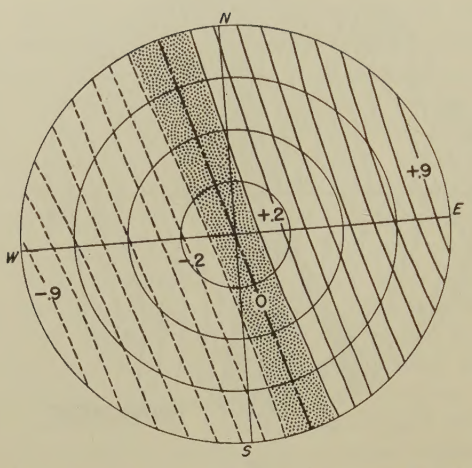

FIG. 5. Set-up for Nantucket Sound. Concentric circles mark intervals of 10 knots. Contour intervals are 0.1 foot.

first case, weather maps showed large high pressure systems over the area, with observed winds at Nantucket not necessarily representative of the larger wind field. In the second case, maps showed deep low pressure systems over Georges Bank and stronger NE winds to the east. It was evident here that dependence on local measured winds for data was limited to the extent in which they were representative of a broad wind field.

An earlier study (Miller 1957) making use of a wind field 300 miles in diameter showed that at Atlantic City, on an open coast without significant promontories or embayments, set-up varied sinusoidally with the direction of wind of a given velocity. This study also showed a quasi-linear relationship between the amount of set-up and increasing velocity of the geostrophic wind. The results of the present analysis for Nantucket Sound indicate a similar sinusoidal variation of sea level and a scale of set-up velocity corresponding to the Atlantic City values.

It was not practicable to determine the general coastal set-up for Montauk or Portland with the data at hand, but it was instructive to apply the generalizations of Atlantic City and Nantucket Sound to both locations. By properly orienting the Atlantic City scale to the coastlines about Montauk and Portland so that maximum general coastal set-ups would be indicated by winds 40° from the normal to the coastline, relations of local set-up were deduced which were consistent with those found about Nantucket Sound.

THE ASYMMETRICAL COMPONENT: THE LOCAL COMPONENT

The contribution of local wind and topography to the set-up observed at the different gauging stations was obtained by subtracting the symmetrical effects attributed to the Sound as a whole from the actual observations. This simple operation gave results for local set-ups which appeared to be systematic and commensurate with the local fetches. Diagrams of local set-up for each of the six tide-gauging stations are shown in Figure 6.

It is instructive to compare the local values of set-up with the open water exposures of each tide gauge (see Fig. 1). Harwichport and Nantucket tide gauges were more or less protected by land barriers from local wind effect except from the southwest for Harwichport and northwest to north for Nantucket. Local set-up showed that Harwichport was vulnerable to excessive sea level from the SSW and Nantucket was vulnerable from the NNW. Lowest local levels were indicated with N winds at Harwichport and SE winds at Nantucket. Other wind directions showed a minimum effect on local level, which was probably due to the limited fetch in these directions. It was apparent that the derived local set-ups agreed qualitatively with the available fetches of each locality.

West Chop and Woods Hole presented modified patterns of local set-up which were more complex than those at the eastern end of the Sound since neither station can be considered a terminus for water transport. Their fetch characteristics from the Sound are mixed with the available fetches in Vineyard Sound and Buzzards Bay.

There were two maxima for positive local set-up in Woods Hole: one from SSW, the Vineyard Sound, and the other from NNW, Buzzards Bay. There were also two maxima for negative set-up: one for SE winds apparently blowing Buzzards Bay water away from Woods Hole, and the other for NW winds affecting Nantucket Sound. In

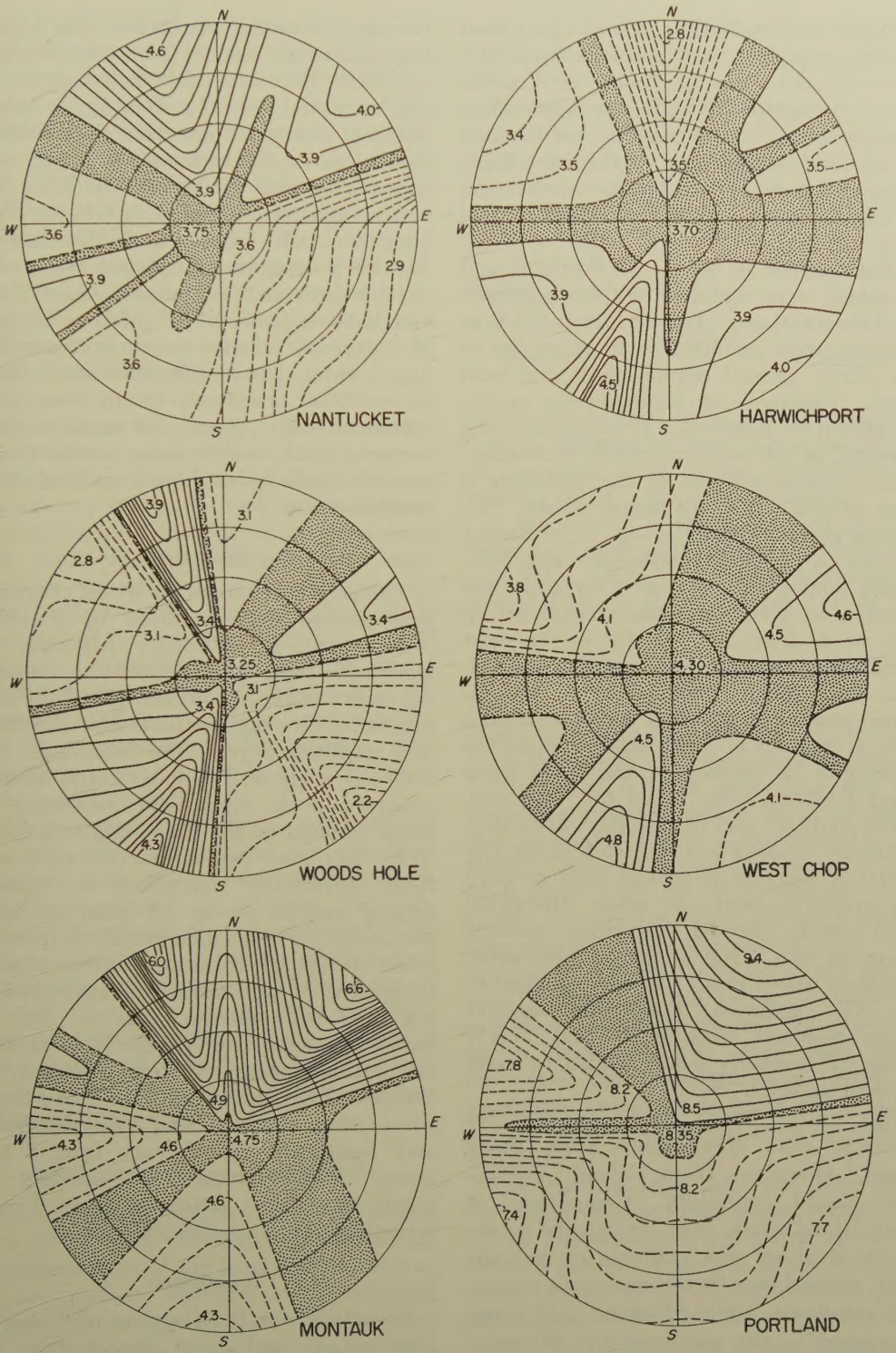

FIG. 6. Local set-ups for six locations, general coastal effects having been removed. Diagrams denote the contribution to total set-up provided by local environment of individual stations and local winds. Concentric circles mark intervals of 10 knots. Contour intervals are 0.1 foot.

contrast to Woods Hole, West Chop local levels appear to be lowered or unaffected by wind from most quarters. Only SSW winds from Vineyard Sound produce appreciable positive set-up. It is possible that the lack of sea level response to wind in the West Chop area is made up for in the acceleration of tidal currents which are notably strong here.

Observations of set-up at Portland and Montauk were matched with appropriate wind observations. The characteristics thus obtained were assumed to be made up of two parts—general coastal effect and local effect. Diagrams of characteristic set-up are shown in Figure 4. It is curious that Portland at the southern end of Casco Bay which itself is exposed to the southeast is least affected with southeast winds. Assuming general coastal set-up to be similar in scale to that at Atlantic City and Nantucket Sound, the Atlantic City scale was oriented to give maximum coastal set-up with winds from 100°, or 40° to the right of normal to the general coastline orientation (050°–230°). Subtraction of the assumed coastal set-up from the observations gave a diagram of local set-up which was reasonable in respect to the local fetch about Portland (Fig. 1 and 6). Portland, at the southern end of box-like Casco Bay, is directly exposed to a NE fetch of the bay itself. A number of islands tend to limit the available fetch in other directions. Local set-up showed maximum positive set-up in the NNE direction and other elevations above mean sea level confined to the NE quadrant. Maximum negative set-up occurred with WSW and WNW winds; all southerly winds tended to lower the local sea level.

The nearest wind data available for Montauk were observations from Block Island. However, Block Island observations lacked continuity. Comparison of Block Island winds with Providence winds, records of which were available for 24 hours a day, showed that Providence was a reasonable substitute. The Montauk tide gauge is exposed to the north on the Montauk peninsula. Characteristic set-ups showed that southerly winds were least effective in raising or lowering sea level. The Atlantic City scale was oriented for a coastline bearing 070°–250° and subtracted from the characteristic set-up. The local set-ups (Fig. 6) derived from this manipulation, showed that maximum positive set-up could be expected with winds from the NE. This corresponds to a line of fetch between Point Judith, Rhode Island, and Montauk. A secondary maximum developed along a line of fetch between New London, Connecticut, and Montauk with NNW winds. The scale of maximum positive set-up was considerably greater than at the other five stations. In contrast, negative set-up was much smaller in scale. Maximum negative set-up occurred with west winds; southerly and easterly winds lowered local sea level only a small amount.

TIME-LAG

The empirical means by which characteristic set-ups were obtained by matching observations of stationary non-tidal sea level with prior winds define the time-lags for each station. However, it is difficult to generalize time lags for particular conditions and localities beyond the following. Statistically a lag of five or six hours between observed peak winds and maximum sea levels applies to the New England Coast. Time lags may vary from one to twenty hours apparently dependent on whether general coastal set-up or local set-up is dominant. This is understandable from the point of view where set-up consists of accumulated wind drift, the greater lag representing drift from greater distance. For instance, time-lags for Woods Hole maxima originating apparently from movement of Buzzards Bay water vary from 2 to 6 hours, while other maxima from Vineyard Sound and Nantucket Sound vary from 5 to 9 hours. These amounts of time-lag are barely more than estimates since the observations show considerable variance.

DISCUSSION

It is clear from the diagrams of characteristic set-up and local set-up that the phenomenon of rise and fall of sea level in response to wind forces is a complex one stem-

ming from local and distant causes. It should not be inferred that these diagrams are complete and finite, since they depend on statistical evaluation for each point of the compass. The nature, duration, and gradient of the winds are such as to indicate that even a statistically correct diagram may not be justifiable for singular events, since it is not known whether the local factors or distant factors will dominate. The interplay of these factors suggests that it would be difficult to generalize for a large area, although that generalization may hold when distant factors prevail.

The storm of November 7, 1953, serves to illustrate to some extent the dominance of one factor over another and the reinforcement or opposition of these factors. Local set-up diagrams were used to obtain the equilibrium condition of local sea level with wind, and the Atlantic City scale (Fig. 5) was used to obtain the general coastal effect. The same observed winds were applied for both the offshore and local equilibria. The results are shown in Figure 7 with the measured non-tidal sea level, barometric effect removed, superimposed over the supposed equilibrium heights. The magnitude of each curve is given in terms of the local tide staff.

It is seen that for Montauk and Portland the local set-up dominated the sea level condition with minor reinforcement from the offshore effects. In contrast, Harwichport and Nantucket were dominated by the offshore factors. Local wind effect served to reinforce or oppose the offshore set-up in a minor fashion. Woods Hole and West Chop were similarly dominated, although it is not clear why West Chop sea level rose in advance of the two conditions of equilibrium with the wind.

FETCH LENGTH AND TOPOGRAPHY

When maximum positive set-up is reached for a given wind velocity and that set-up is representative of the local deformation of the sea surface, equilibrium is assumed between the tangential forces of the wind and sea level. It is necessary to assume, also, that total transport due to wind drift is zero, otherwise the set-up is not truly maximal. It does not follow that the same conditions will hold for maximum negative set-up.

Proudman (1953), in discussing an inclined wind drift in which total transport is zero, introduces a relation where

$$\frac{gh \sin \theta}{u_a^2} = 3.0 \times 10^{-6} \frac{q+3}{q+2}$$

which has bounds of

$$3.0 \times 10^{-6} < \frac{gh \sin \theta}{u_a^2} < 4.5 \times 10^{-6}.$$

Sin θ is the inclination of the sea surface for a given distance or fetch, u_a^2 is the square of the wind force, g the acceleration of gravity, and h is the average depth.

ζ, the amount of set-up, divided by some distance, F, will approximate sin θ provided some fetch length is found corresponding to the total length of deformation. Then

$$3.0 \times 10^{-6} < \frac{g\zeta}{u_a^2} \cdot \frac{h}{F} < 4.5 \times 10^{-6}$$

When the depth-to-fetch ratio is constant, ζ will depend on u_a^2 if equilibrium obtains.

The ratio of depth over fetch sets the theoretical limits of set-up for given velocities of wind. Figure 8 demonstrates the relation between maximum set-up, ζ, and fetch ratio, h/F, for wind velocity of 30 knots. Set-up is given in feet and the fetch ratio is given in feet to nautical miles. The two curves give the limits set by the Proudman function and the circles represent the maximum set-ups indicated by the local set-up diagrams. The large open circle represents the minimum fetch ratio for maximum coastal set-up at Montauk if the path of fetch is taken normal to the general coastline.

The Portland fetch ratio was taken along the length of Casco Bay whose bearing was 15° off the wind direction giving maximum positive set-up. Figure 8 shows Portland set-up for 30 knots of wind and a fetch ratio of 2.3, within the theoretical bounds.

For West Chop the fetch length was rotated 15° from the maximum wind to comply with channel direction of Vineyard

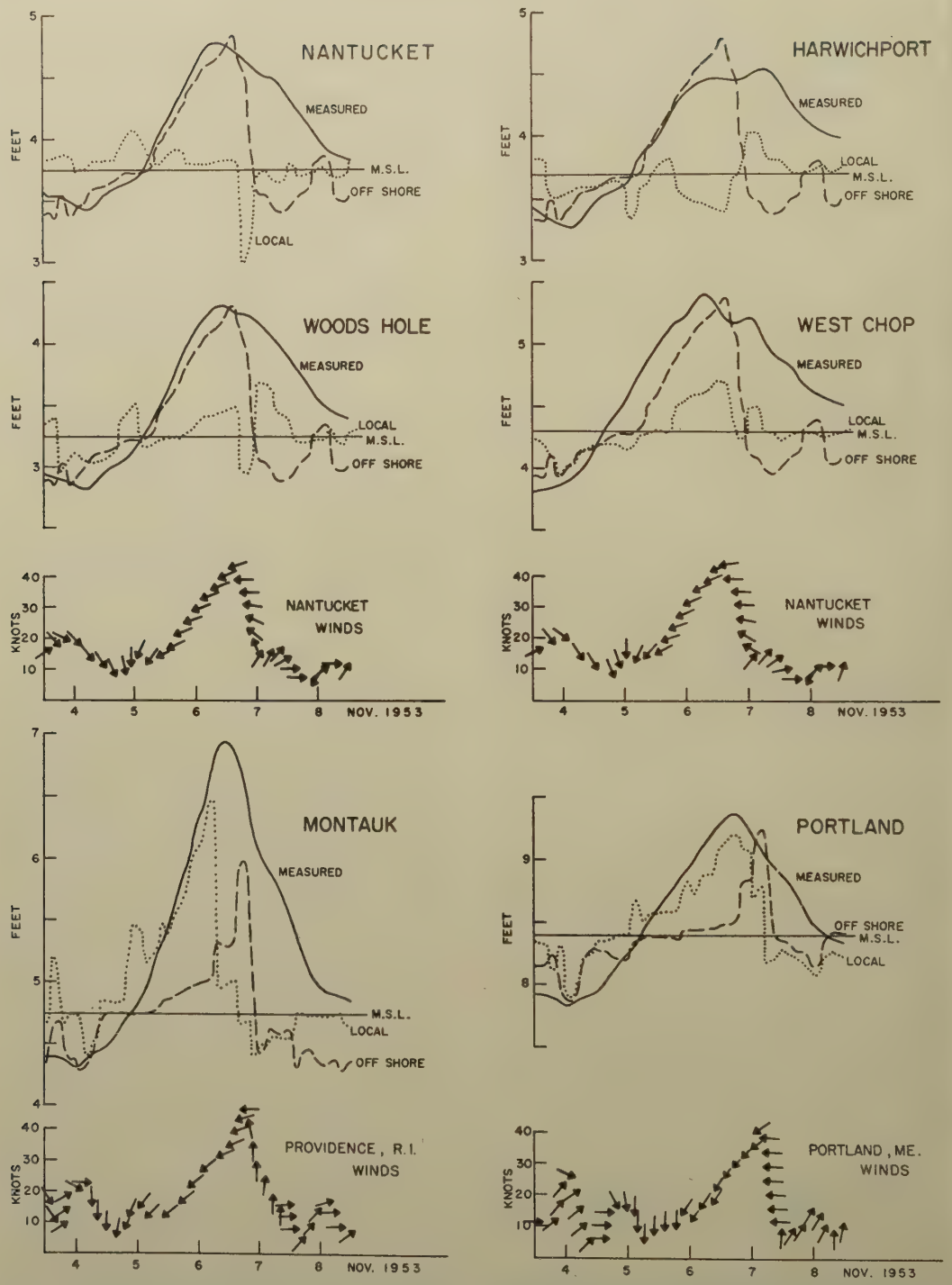

FIG. 7. Comparison of contribution of coastal and local set-ups to the measured non-tidal sea levels for six localities during severe storm of November 7, 1953. Height is given in terms of the tide staff at each locality. Observed winds are shown for each area.

Sound; this was necessary in order to give the wind a water surface to work on. Maximum set-up and a fetch ratio of 5.8 on a much lower scale than Portland, shows a value, for 30 knots of wind, of 0.4 feet, agreeing with the theoretical limit.

The paths of fetch for Portland and West Chop giving maximum local set-up were forced by geographical circumstance into clockwise rotation from the direction of wind. In contrast to the 40° deviation of maximum *coastal* set-up to the right of effective winds, this suggests that the shallower and more limited fetches of local set-up require an angular deviation of approximately 15°. Paths of fetch for the four other stations were taken, allowing for this angular deviation.

Maximum set-up for Woods Hole is greatest with either SSW winds or NNW winds. The first is similar to West Chop with winds piling up water from Vineyard Sound. The second maximum arises from Buzzards Bay. When the path of fetch is rotated from the NNW direction, the fetch runs parallel to the western shoreline of Cape Cod with a depth-to-fetch ratio of 2.1. For 30 knots of wind local set-up is 0.8 feet. The theoretical limits are 0.7 and 1.0 feet.

Maximum local set-up for Montauk arises with NE winds. Angular rotation of path of fetch greatly extends fetch length into the head of Buzzards Bay. The long fetch and shallowness of the Bay give a small ratio of 1.1 and theoretical limits of set-up of 1.1 to 1.8 feet for 30 knots of wind. Local set-up given by the Montauk diagram is 1.6 feet.

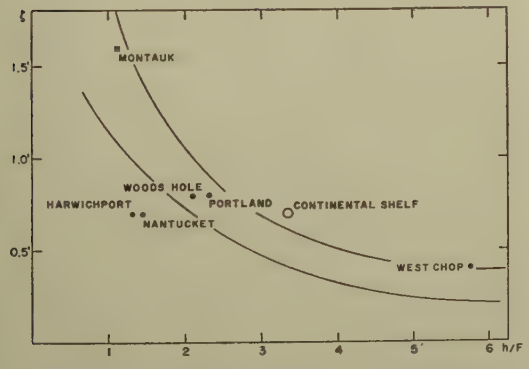

FIG. 8. Set-up as a function of depth-to-fetch ratio. Curves define limits set by Proudman's relation for winds with velocities of 30 knots. Coordinates of the circles are determined from maximum positive set-ups for 30-knot winds and fetches approximately 15° to the right of the wind direction. The units of h/F are feet to nautical miles.

Harwichport and Nantucket set-ups for 30 knot winds do not fall within the theoretical limits. The depth-to-fetch ratios for Harwichport and Nantucket are 1.3 and 1.4 respectively. These are minimum ratios. The path of fetch for Harwichport extends through Muskeget Channel between the islands. If the fetch were taken beyond Nantucket Sound, the deeper water and increasing fetch would increase the ratio. With a shorter fetch the average depth and fetch ratio would also increase, since the shoals of Muskeget Channel would be eliminated from the fetch length. A shorter fetch for Nantucket would increase its ratio also for similar reasons; its fetch may not be lengthened because of the mainland. No alternative numbers are given; however, with fetches five miles shorter than those taken both ratios increase to 2.0 or greater.

On the whole, the maximum local set-ups for all six stations appear to be in agreement with theoretical values if the paths of fetch are based on an approximate 15° deviation to the right of the wind. The fetch for coastal set-up is more obscure, but a ratio which reaches a minimum at the edge of the continental shelf approaches the value of 3 when the path is taken normal to the shelf.

SUMMARY

By adopting a uniform scale for coastal set-up requiring a net transport of water 40° to the right of the wind, an angle of 15° to 20° net drift to the right of wind was developed for local set-up where fetches were limited by local topography. Local effects were separated from distant effects by assuming a sinusoidal or symmetrical variation in the amount of coastal set-up with direction of winds. When the characteristic set-ups for locations about the New England coast were obtained empirically, local set-up

characteristics were derived by removing the effects of coastal set-up from the data. The local set-ups were asymmetrical with the direction of wind, being largely governed by effects of local topography and immediate fetches available. The time-lag between maximum wind and maximum set-up associated with that wind varied between one and 14 hours. A statistical lag of five to six hours is usual for the New England coast; however, actual time-lags are difficult to determine because of the duality of set-up.

REFERENCES

Cline, I. M. 1926. Tropical cyclones. MacMillan, New York. 301 pp.

Doodson, A. T., and H. D. Warburg. 1941. Admiralty manual of tides. His Majesty's Stationery Office, London. 270 pp.

Groves, Gordon W. 1955. Numerical filters for discrimination against tidal periodicities. Trans. Amer. Geophys. Union, **36**(6): 1073–1084.

Miller, Arthur R. 1957. The effect of steady winds on sea level at Atlantic City. Meteor. Monogr., **2**, No. 10: In press.

Proudman, J. 1953. Dynamical oceanography. Wiley, New York. 409 pp.

Redfield, A. C. 1953. Interference phenomena in the tides of the Woods Hole region. J. Mar. Res., **12**(1): 121–140.

Redfield, A. C., and A. R. Miller. 1957. Water levels accompanying Atlantic Coast hurricanes. Meteor. Monogr., **2**, No. 10: In press.

An Approach to Some Problems of Secondary Production in the Western Lake Erie Region

Charles C. Davis

Department of Biology, Western Reserve University, Cleveland, Ohio

ABSTRACT

It has been suggested by Pennak (1955) that tripton, rather than phytoplankton, is the main food of zooplankters in lakes. This concept is based upon his findings that the zooplankton biomass regularly exceeds the phytoplankton biomass, but that the mass of the tripton is greater than that of the zooplankton. In the present study samples of water were gathered from western Lake Erie and from several ponds. These were analyzed for dissolved organic matter, dissolved inorganic matter, volume of phytoplankton, volume of zooplankton, volume of μ-cells (mostly bacteria), and volume of organic and inorganic tripton. The phytoplankton biomass exceeded that of the zooplankton in nearly all samples. The average phytoplankton volume was 4.08 times greater than the average zooplankton volume. Furthermore, the phytoplankton volume usually was greater than that of the organic tripton, the ratio of the average volumes being 2.43 to 1. The reported results support the traditional concept that the zooplankton consumes primarily the phytoplankton. Consideration also is given of the bearing upon this problem of the dynamics of phytoplankton production and of grazing by zooplankters.

In recent years there has been much thinking by limnologists and others on questions of production and productivity in aquatic situations (Dussart 1951, MacFadyen 1948, etc.). These questions are of greatest theoretical interest, for their solution will clarify both the origin of organic material from inorganic (primary production), and the transfer of organic materials through the entire food chain (secondary production). Inasmuch as man himself, through the consumption of fish, etc., is involved in the food chain, these problems are also of great practical interest to those fishery biologists who are concerned with the potential food fish supply from given bodies of water, and with the danger of diversion of the production chain into channels that produce commercially unimportant fish, or that produce no fish at all (*e.g.*, see Anonymous 1954 for a discussion of the production of "trash" fish in Lake Erie).

Both for the ocean (Clarke 1946; Hardy 1956, Chapter 15) and for lakes (Kiefer 1955; Hutchinson, as quoted in Lindeman 1942) it has long been thought that the microscopic planktonic plants form the main base of the food chain, and that these phytoplankters are consumed by planktonic animals, which in turn are consumed by small fish and by some species of larger fish as well (ciscoes, herrings, etc.). Finally, according to this concept, the smaller fish are eaten by larger, predatory fishes, and some of the larger fishes by man. Much of this has been depicted graphically by the familiar food pyramid, as shown, for example, by Juday (1942).

There has been little question but that, with some modification, such relationships as these hold for the oceans, where the fringing shallow water suitable for the growth of attached plants is relatively insignificant, and where the addition from land of consumable organic materials through the action of rains, rivers, and wind is negligible compared to the vast volume of the seas. On the other hand, in lakes and ponds, water appropriate for the growth of rooted plants is much more extensive compared to the total volume of water in the basin, and the influence of organic detritus entering the basin from the temporary or permanent inlets is of much greater importance. Although Forbes' (1887) concept of lakes as self-contained microcosms was very stimulating, even at the time of the reprinting of his paper in 1925 it had become clear that to neglect, as he did, allochthonous influences is un-

realistic, and gives a faulty impression of the simplicity of lake conditions.

Recently Pennak (1945, 1949, 1955) has studied certain Colorado lakes, and has decided that in a "typical" lake (Pennak's quotation marks) the dissolved salts form 75.0 per cent of the constituents of lake water, organic material constitutes 23.1 per cent, tripton 1.2 per cent, zooplankton 0.50 per cent, and phytoplankton 0.15 per cent.

From this basis he concluded that the zooplankton must be consuming the more abundant tripton, rather than the much less abundant phytoplankton. Much earlier, Naumann (1918) arrived at a similar conclusion. On the other hand, Ruttner (1953, p. 146) stated that in alpine lakes where the zooplankton often exceeds the summer standing crop of phytoplankton, the animals "had probably eaten the lakes empty and were living on the fat stored in their bodies." If Pennak's observations are widely applicable, and if his interpretation of his results is correct, it will be necessary to revise sharply our usual concepts of trophic relationships and of the food chain in lakes.

The study reported here was proposed in order to gather data that might throw light upon the above problem. The project was supported by a research stipend provided by the Ohio Division of Wildlife, through the Natural Resources Institute, Ohio State University, Charles A. Dambach, Director. The work was accomplished at the F. T. Stone Laboratory, Put-in-Bay, Ohio. The full and unstinted cooperation of the entire staff of the Stone Laboratory in the provision of working space, materials, field transportation, etc. is gratefully acknowledged. The skiff used during the investigation was purchased through a grant provided by the American Academy of Arts and Sciences.

METHODS

Regular stations were established in the channel between South Bass Island and Middle Bass Island, and in Terwilliger's Pond on South Bass Island. In addition, samples were obtained when possible and convenient at other localities, including Haunck's Pond and Wehrle's Pond on Middle Bass Island, from western Lake Erie off Middle Sister Island, from a quarry on Kelley Island, from the central basin of Lake Erie east of Pelee Island, from East Harbor, etc.

In the shallow ponds, approximately 5-liter samples were obtained from just below the surface. At the deeper stations, samples usually were taken at each meter of depth, and equal portions from each depth were mixed to make a single aggregate sample of approximately 5 liters. A total of 29 samples was collected and analyzed, taken as follows:

1. 6–21–56. Terwilliger's Pond. Surface.
2. 6–21–56. Channel station. 0 and 10 m, mixed.
4. 6–26–56. Terwilliger's Pond. Surface.
5. 6–29–56. Haunck's Pond. Surface.
6. 7–1–56. Channel station. 0 and 10 m, mixed.
7. 7–2–56. East of Pelee Island. 0 and 10 m, mixed.
9. 7–5–56. Terwilliger's Pond. Surface.
10. 7–6–56. Channel station. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 m, mixed.
12. 7–11–56. Wehrle's Pond. Surface.
14. 7–16–56. West of Middle Sister Island. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 m, mixed.
15. 7–17–56. Terwilliger's Pond. Surface.
16. 7–18–56. Channel station. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 m, mixed.
17. 7–23–56. Haunck's Pond. Surface.
18. 7–23–56. Wehrle's Pond. Surface.
19. 7–25–56. Channel station. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 m, mixed.
20. 7–25–56. Terwilliger's Pond. Surface.
21. 7–30–56. Shallow quarry, Kelley Island. Surface.
22. 7–30–56. Deep quarry, east end of Kelley Island. Surface.

23. 8–2–56. Terwilliger's Pond. Surface.
24. 8–3–56. Channel station. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 m, mixed.
25. 8–6–56. Wehrle's pond. Surface.
26. 8–7–56. Terwilliger's Pond. Surface.
27. 8–9–56. East of Pelee Island. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 m, mixed.
28. 8–9–56. Between north end of Pelee Island and Hen Island. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 m, mixed.
29. 8–13–56. Terwilliger's Pond. Surface.
30. 8–14–56. Channel station. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 m, mixed.
31. 8–15–56. East Harbor. 0, 1 and 2 m, mixed.
32. 8–21–56. Channel station. 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 m, mixed.
33. 8–21–56. Terwilliger's Pond. Surface.

Results are not reported fully here from all of these stations. Some were rejected because of breakage, or because of obvious contamination during analysis of weights.

After thorough mixing, aliquot samples were taken from each 5-liter sample for analysis as follows:

1) 1000 ml of filtered water. At first filtration was through a Seitz filter, but this procedure was soon abandoned, whereafter samples were filtered through No. 44 Whatman filter paper under suction. The filtrate was then centrifuged to remove any small particulate material that passed through the filter paper. The filtered and centrifuged water was evaporated to dryness in a weighed evaporating dish on a water bath. The resulting residue was weighed, giving an estimate of the total dissolved solids. The residue was then incinerated in a furnace to burn out the organic materials, and the weight of the resulting ash was determined. The difference between the weight of the solids and the weight of the ash (corrected for carbonates, etc.) was the total dissolved organic material.

2) 200 ml was placed in a tall cylinder, preserved with Lugol's solution as modified by Rodhe, and the plankton and detritus allowed to settle. After decantation of the supernatant water until the particulate materials were concentrated into only 10 or 15 ml, quantitative analyses were made of the phytoplankton, zooplankton, and detritus, the species of animals and plants being determined wherever necessary and possible. The volumes of the several categories were determined by careful measurements in the usual manner with ocular micrometers and with the calibrated fine adjustment of the microscope. The organisms or bits of detritus were considered as spheres, cones, cylinders, cubes, or other appropriate shapes. Diffuse small particles of decaying detritus were dealt with, where accuracy allowed, by estimating the proportion of the total occupied by water spaces. Where such estimates were not possible, the individual portions of the detrital masses were measured separately, and summed. Volumes are expressed in cubic decimillimeters ($1 dmm^3 = 10^6 \mu^3$).[1]

It was recognized that there would have been definite advantages if comparisons were made using dry weights of zooplankton, phytoplankton, and detritus rather than volumes, inasmuch as the volume measurements did not take into consideration an unknown content of water that undoubtedly varied irregularly from one category to another, or even from one species of plant or animal to another. However, the technical difficulties of physically separating these three categories to determine their dry weights could not be solved, whereas the categories could be separated optically to determine volumes.

It is inevitable that certain errors of measurement have crept in, although every possible effort was made to minimize and standardize them. The volumes given should be considered as approximations only, but it is felt that they are preferable

[1] The volumetric unit dmm^3 was suggested for use in this paper by Dr. D. G. Frey of Indiana University.

(until more precise methods are devised) to no measurements at all.

In order to make rough comparisons of the results given below with those given gravimetrically by many other authors, it should be said that 1 dmm^3 of pure water weighs 1 mg, and hence 1 dmm^3 of plankton or organic detritus has a wet weight of somewhat more than 1 mg (the average specific gravity being rather greater than that of water). If we assume that the plankton and the organic detritus average 90 per cent water and 10 per cent solids, then the dry weight of 10 dmm^3 is approximately 1 mg (conversely, 1 mg of dissolved organic matter is roughly equivalent to 10 dmm^3 of plankton or organic detritus).

3) 500 ml was preserved with 4 per cent formaldehyde, and centrifuged with a Foerst centrifuge until the particulate material was concentrated into 10 or 15 ml, and analyses of plankton and detritus were made (as described under 2).

It is intended to make detailed comparisons of the results obtained by these two methods of preservation and concentration, to ascertain their respective merits as methods of analysis. This comparison will not be detailed below. It is apparent from the results that the analyses of the samples preserved with Lugol's solution reflect the actual quantities of plankton present more accurately, and only these will be used here.

4) 100 ml samples were titrated with a standard solution of H_2SO_4, using phenolphthalein and methyl orange as indicators, in order to determine the alkalinities, and hence to estimate the carbonate and bicarbonate content of the water (see American Public Health Association 1946, pp. 9, 31–32).

RESULTS

1. Alkalinities

Only two of the tested samples showed any phenolphthalein alkalinity, and in both of these it was very small. In the open lake and in Terwilliger's Pond the methyl orange alkalinity was rather uniform, varying from 102 to 111 ppm $Ca(HCO_3)_2$, expressed as $CaCO_3$. Samples from Wehrle's Pond were significantly higher (122 and 123 ppm), and from Haunck's Pond the only sample tested was relatively very high (186 ppm). Samples from two quarries on Kelleys Island had around the same alkalinity as Wehrle's Pond (namely, 121 and 127 ppm). In a single sample from East Harbor the alkalinity was only 98 ppm. The alkalinities that were obtained are shown in Table 1.

Many determinations of alkalinities have

TABLE 1. *Alkalinities for samples tested*

Sample No.	Location	ppm $CaCO_3$	
		Phenolphthalein	Methyl orange
14	W. of Middle Sister Island	0	105
15	Terwilliger	4	111
16	Channel	0	107
17	Haunck	0	186
18	Wehrle	0	123
19	Channel	0	108
20	Terwilliger	4	107
21	Shallow quarry	0	121
22	Deep quarry	0	127
23	Terwilliger	0	109
24	Channel	0	109
25	Wehrle	0	122
26	Terwilliger	0	108
27	East of Pelee Island	0	106
28	East of Hen Island	0	103
29	Terwilliger	0	102
30	Channel	0	107
31	East Harbor	0	98
32	Channel	0	103
33	Terwilliger	0	107

TABLE 2. *Some previous alkalinity determinations from Lake Erie, as ppm $CaCO_3$*

Author	Locality	Range	Average
Anderson (1946)	Western basin	104 –124*	114*
Chandler (1940, 1942, 1944)	Western basin	80 –96.9	
Gottschall and Jennings (1933)	Presque Isle Bay and adjacent lake		93.6
Lenhardt (1955)	L. Erie		100
Leverin (1947)	L. Erie	92.5–100.0	
McMillan and Verduin (1953)	Western basin	90	
Meyer *et al.* (1943)	Western Basin	79.8–84.0	
Ohio Div. Water (1953)	Western and central basins	70 –160*	113*
Wagner (1929) and Williams (1929)	Eastern basin	62 –118	94.8
Wright *et al.* (1955)	Western basin	85 –179	97
	(Island section)	85 –103	91

* Erroneously (?) reported as ppm HCO_3^-.

been made in the past for Lake Erie. Some of the more recent among them are given in Table 2. It will be noted that most of the alkalinities are lower than those obtained in the open lake waters in the present study, with the exception of the results reported by the Ohio Division of Water (1953) and by Anderson (1946) where the averages (113 and 114 ppm) compare very closely with my average of 107 ppm. Comparative figures for the ponds, the quarry, and East Harbor apparently have never been published.

On the basis of the above determinations of alkalinities, the weights of samples that had been dried and incinerated were corrected. During incineration of the dried samples, the carbonates and bicarbonates were transformed into oxides:

$$Ca(HCO_3)_2 \rightarrow CaO + 2CO_2 + H_2O$$
$$(122 \text{ g } HCO_3^- \rightarrow 16 \text{ g } O^=)$$

$$CaCO_3 \rightarrow CaO + CO_2$$
$$(60 \text{ g } CO_3^= \rightarrow 16 \text{ g } O^=)$$

Without the necessary corrections, the values obtained for the organic and inorganic content of the water would be faulty.

At first alkalinities were not determined. In these early cases, average values for the rest of the determinations were used (107 ppm for open Lake Erie water, and for Terwilliger's Pond; 186 ppm for Haunck's Pond; and 123 ppm for Wehrle's Pond). Corrections were made by adding calculated amounts to the weights of the incinerated samples. Apparently such corrections often are not made in the estimation of the organic content of water by the incineration method (Welch 1948, p. 295), so that many determinations of dissolved organic matter as reported in the literature are too high (*e.g.*, in Birge and Juday 1934, and Dineen 1953; on the other hand, Hooper 1951 and Pennak 1955 recognized this source of error and made corrections for it).

2. Dissolved organic matter

Dissolved organic matter (see Table 3) varied from 39.5 to 87.0 mg/L, with an average for 19 determinations of 70.6 mg/L (72.3 if sample No. 1, which was much lower than any of the others and which therefore may be faulty, is omitted). Samples obtained (Nos. 17–33) later in the season when blue-green algae were more

TABLE 3. *Dissolved and particulate materials*

Sample No.	Diss. org. mg/L	Diss. inorg. mg/L	Phytoplankton dmm³/L	Zooplankton dmm³/L	μ-cells dmm³/L	Org. detritus dmm³/L	Inorg. detritus dmm³/L
1	39.5	142.5	7,641	73	203	3,714	260
2	71.9	100.2	10,332	68,303	18	1,555	1,072
4	81.1	103.7	23,371	1,861	81	9,108	587
5	76.5	133.5	49,875	1,905	102	353	274
6	74.4	108.9	2,235	144	78	2,797	537
7	65.1	108.4	1,331	739	48	3,590	395
9	—	—	5,400	1,487	75	13,731	318
10	—	—	2,311	593	10	2,958	259
12	59.6	101.4	6,886	1,258	417	12,896	550
15	62.4	103.3	19,586	2,911	280	9,427	340
16	65.3	96.7	4,477	219	25	4,030	89
17	82.3	147.5	47,716	1,539	154	8,626	441
19	74.8	95.8	4,725	5,028	43	2,906	261
22	55.5	185.7	19,578	874	11	233	70
25	77.9	91.8	50,064	6,047	338	11,422	751
26	69.5	96.9	7,540	651	259	5,855	390
27	67.3	97.0	3,168	483	11	3,163	31
29	69.1	88.6	5,474	2,076	67	23,923	199
30	75.5	91.4	20,219	329	39	5,444	223
31	87.0	91.9	88,934	435	290	31,022	2,204
33	86.8	87.2	5,796	745	48	7,429	228
Av	70.6	109.2	18,989	4,652	124	7,818	451

abundant averaged 76.7 mg/L. The fact that this value is somewhat higher than the overall average for the season agrees with the common observation that the blue-green algae, when abundant, are associated with increased quantities of dissolved organic matter. Many authors, such as Rodhe (1948), believe that the organic material is required for the growth of the blue-green algae, although Gerloff *et al.* (1950), on the basis of laboratory experiments, concluded that it is not needed. From the present data there is no indication whether the increase in the numbers of blue-green algae is caused by the increase in quantity of dissolved organic mtter, or whether the increase of organic matter is caused by the abundant blue-green algae.

The average dissolved organic matter for the two samples from Haunck's Pond was 79.4 mg/L; that for two samples from Wehrle's Pond was 68.8 mg/L; that for 6 samples from Terwilliger's Pond was 68.1 mg/L (If a single determination from Terwilliger's Pond of 39.5 mg/L, which is much lower than any other determinations from any locality, is rejected as being probably incorrect, the average for Terwilliger's Pond was 80.9 mg/L.); and that for 7 samples from the open lake was 70.6 mg/L. Single determinations from the deep quarry on Kelley Island, and from East Harbor were respectively 55.5 and 87.0 mg/L.

The total dissolved organic content of lake waters has been reported only infrequently, most authors confining themselves to measurements of B.O.D., organic C, organic N, etc. Perhaps the most extensive investigations were those of Birge and Juday (1926, 1934) on a large number of lakes in Wisconsin. In their lakes they reported dissolved organic matter ranging from 3.26 to 55.34 mg/L. All of these results are well below the average obtained in the present study. A few other authors have reported total organic matter. Dineen (1953) measured 108.2 to 311.6 mg/L. Dissolved organic matter was determined by Pennak (1945, 1949) in several Colorado lakes and reservoirs. Most of his results were lower than those reported here, even down to 1.90 mg/L, but some ranged as high as 1210 mg/L.

3. Dissolved inorganic matter

Dissolved inorganic matter (see Table 3) varied from 87.2 to 185.7 mg/L, and averaged 109.2 mg/L. Towards the end of the season there was less inorganic material dissolved in the water than earlier in the year. The highest values occurred in the deep quarry on Kelley Island (185.7 mg/L) and in Haunck's Pond on Middle Bass Island (average of two samples was 140.5 mg/L).

4. Phytoplankton

The following phytoplankters were recognized for the purpose of enumeration and calculation of volume:

Blue-green algae:

Anabaena
Anacystis (*Microcystis*)
Aphanizomenon flos-aquae
Aphanocapsa
Chroococcusa
Merismopedi
Oscillatoria
Spirulina

Diatoms:

Asterionella
Fragillaria sp.
F. crotonensis
Melosira
Pleurosigma
Stephanodiscus (+*Cyclotella*)
Synedra
Tabellaria

Green algae:

Actinastrum
Ankistrodesmus
Coelastrum
Crucigenia
Lagerheimia
Oöcystis
Pediastrum boryanum
P. duplex
P. simplex
Scenedesmus
Schroederia
Tetraedron

Desmids:

Closterium
Cosmarium
Staurastrum

Dinoflagellates:

Ceratium hirundinella
Glenodinium
Peridinium sp.
P. gatunense

Other holophytic flagellates:

"flagellates"
Dinobryon
Euglena
Pandorina
Phacus

Other algae occurred from time to time and from place to place, but they were encountered only in small numbers. They would have had no essential effect upon the total phytoplankton volumes, and hence they were not enumerated.

As can be seen in Table 3, phytoplankton volumes varied from 1,331 to 88,934 dmm^3/L, with an average of 18,989. The lowest average volumes were encountered in samples from the open lake, where the average of 8 samples was 6,100 dmm^3/L. The next lowest average was found in Terwilliger's Pond (which is little more than a semi-enclosed arm of the lake), with 11,211 dmm^3/L. Greatest volumes occurred in Haunck's Pond, where the average of two samples was 48,795 dmm^3/L, and Wehrle's Pond, with an average of 28,476 for two samples. The one sample analyzed from East Harbor contained 88,934 dmm^3/L. This sample was obtained at the height of the blue-green algae season. The value of 19,578 dmm^3/L for the deep quarry on Kelley Island probably is too high for purposes of comparison with the others for two reasons: 1) the cells of all the phytoplankters were chlorotic, and many were distinctly moribund, so that it was difficult to distinguish living from deceased cells, and 2) the sample could be obtained only from the surface, whereas the quarry was perhaps 10 m deep. Phytoplankters may have been confined to the upper layers, and a mixed sample from several depths might very well have presented a different picture upon analysis.

Phytoplankton volumes have previously been determined in Lake Erie by other investigators. Verduin (1951a) reported phytoplankton volumes from 1,120 to 21,600 dmm^3/L during the 1949 spring diatom maximum in western Lake Erie (by his mobile sampling method). In the same paper he reported a minimum of 960 dmm^3/L near the surface at a station near Put-in-Bay in December 1948. In the same vicinity, during July 1949 he found from 5,120 to 9,120 dmm^3/L. In subsequent papers, the same author (Verduin 1951b, 1954) compared the average of 16,480 dmm^3/L at the peak of the 1949 vernal phytoplankton bloom with an average of only about 3,000 dmm^3/L in 1950, 1,000 in 1951, 2,000 in 1952, and 1,000 in 1953.

Sullivan (1953) listed phytoplankton volumes near the mouths of several rivers in the central and western basins of the lake at various dates in 1950–1951. He found from 848 dmm^3/L off the Ashtabula River to 2,924 dmm^3/L off the Vermillion River. Hintz (1955) indicated from 600 to 1,700 dmm^3/L in the island region of western Lake Erie in the fall of 1952 and the spring of 1953. In the central basin of the lake, off Cleveland, Davis (1954a) estimated phytoplankton volumes during the year from September 1950 through September 1951 ranging from a minimum of 3 dmm^3/L in June to a maximum of 2,815 dmm^3/L in April.

The results obtained in the present investigation from Haunck's, Wehrle's, and Terwilliger's Ponds, and from East Harbor and the Kelley Island quarry cannot be compared to any published reports for the area. The water of Haunck's and Wehrle's Ponds was obviously very rich in phytoplankton, for the samples were cloudy and green due to the presence of large numbers of blue-green and green algae. The single sample from East Harbor had a high phytoplankton volume primarily because of the presence of large numbers of blue-green algae.

It will be noted that all of the previously reported phytoplankton volumes from Lake Erie itself are considerably exceeded by the present results except the values estimated by Verduin for the spring bloom of 1949. The average value for the open lake in the present study is of about the same order of magnitude (6,100 dmm^3/L) as the average volume reported by Verduin (1951a) for July of 1949, namely, 5,547 dmm^3/L.

In comparing the results reported here with others, it should be kept in mind that the previous studies were of samples obtained by filtering known volumes of water through small bolting silk discs (Verduin, Sullivan, Hintz) or by filtering through the small cone-shaped net of a Juday plankton trap (Davis). By these methods the nannoplankters are lost to a greater or lesser extent, and hence the estimations that are obtained are undoubtedly somewhat low. In the present study the samples were allowed to settle by gravity after being preserved with a modified Lugol's solution. The I_2-KI-acetic acid solution allowed the settling of even the floating species of blue-green algae, and settling was continued

sufficiently long to insure the complete settling of even the tiniest nannoplankters. Hence it was felt that, within the limits of errors inherent in the methods of sampling and counting, the results reflect reality comparatively closely. Details will be reported elsewhere later, but it should be mentioned here that corresponding aliquots of each sample were preserved with formaldehyde and were centrifuged and analyzed. Results almost invariably were significantly lower than in the Lugol-preserved aliquots, especially for those species of blue-green algae that tend to float on the surface (for these are almost completely lost in the centrifuge).

5. Zooplankton

The following categories of the zooplankton were distinguished for the purpose of counting numbers and estimating volumes:

Protozoa:

Arcella
"ciliates"
Codonella cratera
Difflugia
Epistylis
Tintinnopsis
Vorticella

Rotifers:

Asplanchna
Conochilus
Filinia
Kellicottia longispina
Keratella cochlearis
Polyarthra
Synchaeta
Trichocerca
unidentified rotifers
rotifer eggs

Copepods:

nauplius
juvenile *Cyclops*
Cyclops adults
juvenile *Diaptomus*
Diaptomus adults

Cladocerans:

Bosmina
Chydorus
Daphnia longispina
juvenile *Daphnia retrocurva*
Daphnia retrocurva

For two reasons it is felt that the estimations of volume of the larger zooplankters are less reliable than the estimations of phytoplankton volumes and the volumes of the smaller zooplankters. Firstly, the larger animals naturally occurred in smaller numbers per unit volume of the lake water, and hence were not sampled as adequately as were the smaller forms. Secondly, the volumes (of the Cladocera especially) were more difficult to estimate accurately because of the irregularity of the bodies and appendages. It seems likely that cladoceran volume estimates were somewhat too high, although this does not invalidate the results, as will be discussed below.

Estimations of zooplankton volumes for particular samples (see Table 3) ranged from 73 dmm^3/L in Terwilliger's Pond on June 21 to 68,303 dmm^3/L at the channel station on the same date. The average for all samples analyzed was 4,652 dmm^3/L. In the open lake the average was 9,480 dmm^3/L. Included in this average, however is one sample (68,303 dmm^3/L) which is 10 times as large as the next smaller sample from any location. Excluding this sample, the average for the remainder of the open lake samples was 1,077 dmm^3/L. The average for all the samples analyzed from Terwilliger's Pond was about the same as the average from the open lake (exclusive of the one large volume), namely 1,401 dmm^3/L. In Haunck's Pond the average value of 1,722 dmm^3/L was somewhat higher, while in Wehrle's Pond the average was three times as large, or 3,653 dmm^3/L. In the quarry and in East Harbor, zooplankton volumes were smaller, 874 and 435 dmm^3/L, respectively.

Determinations of zooplankton volumes apparently have not previously been published for Lake Erie or the other Great Lakes. Hence no comparisons can be attempted. Comparisons of the number per liter determined here with the quantitative estimates reported by Chandler (1940), Davis (1954b), and others would contribute little to an understanding of the problems under consideration.

6. "μ-cells"

Invariably in the samples there were a number of tiny cells, which were designated μ-cells, following Rodhe's (1955) use of the term "μ-algae." Apparently most of these were bacteria, but some were single cells of blue-green algae. They averaged, according to a series of estimates, approximately 1 μ in diameter. Although as individuals they were extremely numerous at times, *in toto* they never formed large volumes. Total volumes, as seen in Table 3, ranged from 10 dmm^3/L at the channel station to 417

dmm^3/L in Wehrle's Pond. The average volume for all localities was 124 dmm^3/L. In the open lake the average was rather low, namely 34 dmm^3/L. Only the quarry, with 11 dmm^3/L (a single sample), contained a smaller volume. At the other extreme, Wehrle's Pond had an average μ-cell volume of 378 dmm^3/L. In Terwilliger's Pond and Haunck's Pond the averages more nearly approximated the over-all average, with 145 and 128 dmm^3/L, respectively. The single determination from East Harbor showed 290 dmm^3/L.

The quantity of μ-cells was not always closely proportional to the quantity of organic detritus, as can be seen from Table 3. All of the 5 lowest values obtained for μ-cells (< 25 dmm^3/L), however, are associated with low values ($< 4{,}100$ dmm^3/L) of organic detritus, and the 5 largest volumes of μ-cells (> 250 dmm^3/L) are all associated with higher organic detritus values ($> 5{,}800$ dmm^3/L). On the other hand, there are several instances of large volumes of μ-cells in conjunction with small volumes of detritus, and *vice versa*. Despite such discrepancies, however, the coefficient of correlation between μ-cell volumes and volumes of organic detritus was calculated to be 0.466, which is significant at the 5 per cent level (19 degrees of freedom). Similar conclusions apply to correlations between μ-cell volumes and quantities of total seston. Assuming that most of the μ-cells are bacteria, these observations support the usual contention that there is a relationship in the pelagic zone between bacterial numbers and the quantity of suspended matter in the water. Some of the contradictory evidence on this relationship is reviewed by Kuznetsov 1954.

7. Organic detritus

The distinction between organic and inorganic detritus was made in a more-or-less subjective manner. Often, the remains of cellulose cell walls could clearly be recognized as organic. Sand grains, the silicious tests of diatoms, etc. clearly were inorganic. Most materials, however, appeared to be organic matter in various stages of decay, and these were listed as organic, even though it was not always absolutely certain that they were so.

The volumes of organic detritus (as shown in Table 3) varied from 233 to 31,022 dmm^3/L, with an average of 7,818. The average volume for the open lake was 3,305 dmm^3/L, that for Haunck's Pond was 4,490, that for Terwilliger's Pond was 10,455, and that for Wehrle's Pond was 12,159 dmm^3/L. The single samples analyzed from East Harbor and from the quarry contained respectively the smallest and the largest volumes of organic detritus, namely 233 and 31,022 dmm^3/L.

8. Inorganic detritus

Inorganic detritus, as a rule, was present in much smaller quantities than organic detritus. Volumes ranged from 31 dmm^3/L east of Pelee Island in the central basin of the lake, to 2,204 dmm^3/L in East Harbor. The average was 451.

Inasmuch as the amount of inorganic detritus has little direct bearing upon the problems being dealt with here, averages for the several localities will not be given (see Table 3).

DISCUSSION

From the results given above and from the comparisons given in Figure 1*A*, it can be seen that the average phytoplankton volume was 4.08 times as large as the average zooplankton volume. The average phytoplankton volume in Haunck's Pond was 28.34 times as large as the average zooplankton volume. In Wehrle's Pond it exceeded the average zooplankton volume 7.82 times. For Terwilliger's Pond the comparable figure was 8.00 times, while for the open lake (exclusive of the large value in one sample) it was 5.66 times (including the large value, the average zooplankton volume exceeded the phytoplankton volume 1.55 times). In the quarry and at East Harbor, where only single samples were analyzed, the phytoplankton volume was respectively 22.40 and 204.45 times as large as the zooplankton volume.

Furthermore, the average phytoplankton volume exceeded the volume of organic detritus 2.43 times, while the latter exceeded the zooplankton volume 1.68 times. Figure 1*A* shows that the average dissolved inorganic material is 1.55 times greater than the av-

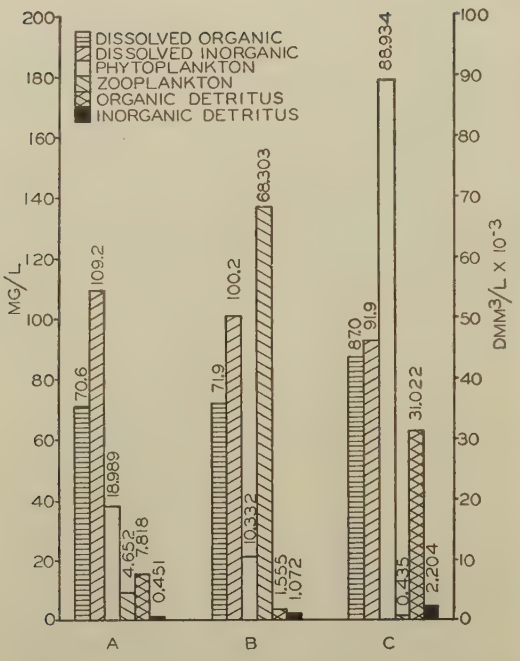

FIG. 1. Comparisons among dissolved organic matter (mg/L), dissolved inorganic matter (mg/L), phytoplankton (dmm³/L), zooplankton (dmm³/L), organic detritus (dmm³/L), and inorganic detritus (dmm³/L). *A*. Averages for all samples. *B*. Sample from the channel station, showing maximum zooplankton volume. *C*. Sample from East Harbor, showing maximum phytoplankton volume.

erage dissolved organic. This differs from the situation obtained by Pennak, where the dissolved inorganic matter was 3.25 times greater than the dissolved organic (however, the amount of dissolved inorganic matter varies greatly from lake to lake, and hence there is little significance in ratios of organic to inorganic except as a reflection of this variability).

Considering each sample individually, similar results appear. With some variations of proportions, the phytoplankton volume usually was greater than either the zooplankton or the volume of the organic detritus. The two samples showing the greatest extremes are figured. Figure 1*B* depicts the comparative results obtained from the sample collected in the channel on June 21, when the zooplankton volume was greater by 10 times than in any other sample. The great quantity of zooplankton was not, apparently, caused by a temporary and/or local swarm of zooplankters, as might be suspected, for during this period it was observed that the Cladocera were extremely abundant in the water for over a week, both during the day and during the night in the channel and in nearby Hatchery Bay. Because the bulk of the zooplankters were Cladocera, and because (as explained above) the estimated volumes of the Cladocera were suspected to be too large, it is probable that the reported zooplankton volume should not be as great as is indicated. On the other hand, it is not considered that estimates of Cladoceran volumes exceeded reality by more than a factor of 2, and therefore the conclusions that are drawn still would be valid. The volume of the zooplankters exceeded the combined volumes of the phytoplankton and of the organic debris 5.7 times in this sample. It seems probable that the large volumes of zooplankton in the early portion of the season were (ecologically) based largely upon the vernal phytoplankton maximum. This could not be shown in the results, however, for the vernal pulse had passed before the study could begin. The larger zooplankters have a slower developmental history than phytoplankters and smaller zooplankters (Davis 1954b, Ruttner 1952: 145, Steeman Nielsen 1937) and therefore tend to lag behind their food plants in abundance.

Figure 1*C* shows the results obtained from the sample collected in East Harbor, where the phytoplankton volume was at its maximum for all the samples analyzed. Zooplankton was very poorly developed, and was greatly exceeded, not only by the phytoplankton, but by the organic detritus as well.

It is believed that it is necessary to place major emphasis in the present paper on average results, rather than placing too much stress on the results obtained from single samples, because each sample was taken from a population whose previous history and present trend was unknown. However, some relationships of interest appear from Figure 2, where the samples are considered individually. In the graph the samples are arranged in the order of their decreasing phytoplankton volumes per liter, and comparisons are made among the volumes of phytoplankton, zooplankton, and

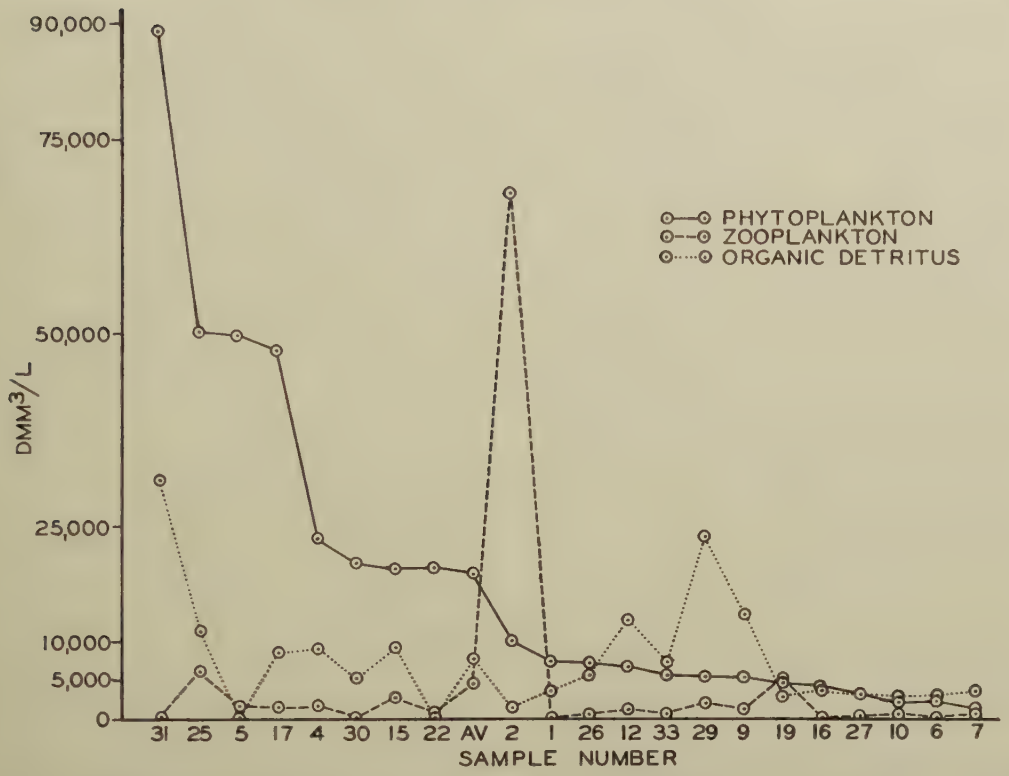

FIG. 2. Volumes of phytoplankton, zooplankton, and organic detritus for the individual samples, arranged in order of decreasing phytoplankton volumes.

organic detritus. Following are some of the more obvious relationships:

1) The greatest volume of organic detritus occurred in the sample with the greatest volume of phytoplankton, although only about ¼ less detritus occurred in another sample where the phytoplankton was only 0.062 times as great.

2) The greatest volume of the zooplankton occurred in a sample with a low-intermediate quantity of phytoplankton, but the lowest volumes of phytoplankton (right end of the graph) are associated with the lowest volumes of zooplankton.

3) At no time is there a clear and consistent relationship between the zooplankton volume and that of the organic detritus. It might have been suspected that the zooplankton would consume the detritus to a greater or lesser extent, hence decreasing its quantity. At the same time, it might have been suspected that the zooplankters would produce numerous fecal pellets, which themselves would become part of the detritus, increasing the detrital volume. However, the higher volumes of zooplankton are not consistently associated with high, nor low, nor intermediate volumes of detritus, probably because of the varying origins of the detritus in the several samples, and because of differing effects of different kinds of zooplankters.

4) Only in 5 of the 21 samples is the volume of detritus very much greater than that of the phytoplankton, and the average detrital volume for all of the samples is only 41.2 per cent of the average phytoplankton volume.

There is little in the results obtained in the present study that supports the conclusions of Pennak (1955) that there is a consistent superiority of zooplankton over the phytoplankton, and of the tripton over the zooplankton, or that the zooplankton must consume largely tripton rather than phytoplankton. Only one sample in the

present series had an appreciably greater zooplankton volume than phytoplankton, and in this sample there was much less tripton than phytoplankton. Possibly the basis for the contrary results obtained by Pennak and myself lies in the differences in the bodies of water studied, and in the methods. Pennak's methods differed considerably from those used in the present study for deriving comparative values of the zooplankton, phytoplankton, and tripton. (In his 1955 paper he stated that the tripton was estimated to be 65 per cent of the seston, the figure being a generalization from "rough measurements of the size and volume of many plankton organisms as compared with the total seston determinations." Seston was determined by weighing the dried centrifugate from one liter of sample.)

Two further considerations appear to be rather important. Firstly, in a lake, tripton can be assumed to have four major sources: 1) from detritus washed into the lake from the shore or from the inlets of the lake, 2) from decayed attached vegetation growing in the shallow portions of the lake, 3) from the dead and decaying bodies of plankters, and 4) from sediments resuspended from the bottom by wave and current action. In ordinary temperate lakes in the warm season the main source of tripton probably is the 3rd of the above sources, for decay of the attached plants occurs mainly in unfavorable seasons, the run-off is mainly in spring, or sporadically at other times, and wave action is less violent as a rule in the summer and early fall. If this be true, then there is relatively little value in discussing the proportional quantities of phytoplankton and of tripton used as a food source by the zooplankters, for both these categories have the same ecological origin.

Secondly, the rate of turnover of the phytoplankton, especially of the nannoplankton, in many respects is more important than the standing crop. Yet usually it is only the standing crop that can be measured. Conclusions frequently are reached that neglect the dynamics of plankton production. These matters have been thoroughly reviewed recently by Edmondson (1957).

Pennak (1946, and personal communication) is thoroughly aware of the dynamics of the phytoplankton turnover. In his paper (1955), however, it would appear that he came to conclusions concerning the relations between the zooplankton and the phytoplankton without full consideration that the zooplankters might be consuming small phytoplankters rapidly. Such a rapid consumption by zooplankters of a rapidly reproducing phytoplankton population could result in a high ratio of the zooplankton standing crop to the phytoplankton standing crop (high $Z:P$ ratio). This would not necessarily imply that the zooplankters were therefore consuming tripton, even though this were more abundant than the phytoplankton. As suggested by Edmondson (1957), the large quantities of detritus in such circumstances may simply be caused by the production of a copious number of fecal pellets by zooplankters that are well fed through their consumption of phytoplankton.

It should not be inferred from the above that the present author denies the importance of allochthonous influences or of non-phytoplankton autochthonous influences, for the opposite is the case. It merely appears from the results reported in this paper, and from general considerations, that it is not proper to put greater emphasis upon the utilization of detritus by zooplankters than is warranted by available information. These matters, however, need much further investigation. It is hoped to delve more into them in the future, both in the Great Lakes and in smaller lakes and ponds.

REFERENCES

American Public Health Association. 1946. Standard methods for the examination of water and sewage. 9th ed. New York. pp. i–xvii, 1–286.

Anonymous. 1954. Unused resources of Lake Erie. Progr. Fish-Cult., **16**(4): 181.

Anderson, Bertil G. 1946. The toxicity thresholds of various sodium salts determined by the use of *Daphnia magna*. Sewage Works J., **18**(1): 82–87.

Birge, E. A., and C. Juday. 1926. Organic content of lake water. Bull. U. S. Bur. Fish., **42**: 185–205.

———. 1934. Particulate and dissolved organic matter in inland lakes. Ecol. Monogr., **4**(4): 440–474.

Chandler, David C. 1940. Limnological studies of western Lake Erie. I. Plankton

and certain physical-chemical data of the Bass Islands region, from September, 1938, to November, 1939. Ohio J. Sci., **40**(6): 291–336.

———. 1942. *Ibid.* III. Phytoplankton and physical-chemical data from November, 1939 to November, 1940. Ohio J. Sci., **42**(1): 24–44.

———. 1944. *Ibid.* IV. Relation of limnological and climatic factors to the phytoplankton of 1941. Trans. Amer. Micr. Soc., **63**: 203–236.

Clarke, George L. 1946. Dynamics of production in a marine area. Ecol. Monogr., **16**(4): 321–335.

Davis, Charles C. 1954a. A preliminary study of the plankton of the Cleveland Harbor area, Ohio. II. The distribution and quantity of the phytoplankton. Ecol. Monogr., **24**(4): 321–347.

———. 1954b. *Ibid.* III. The zooplankton and general ecological considerations of plankton production. Ohio J. Sci., **54**(6): 388–408.

Dineen, Clarence F. 1953. An ecological study of a Minnesota pond. Amer. Midl. Nat., **50**(2): 349–376.

Dussart, B. H. 1951. La productivité de l'eau. Hydrobiologia, **3**(4): 331–356.

Edmondson, W. T. 1957. Trophic relations of the zooplankton. Trans. Amer. Micr. Soc., **76**(3): 225–245.

Forbes, Stephen A. 1887. The lake as a microcosm. Bull. Illinois State Nat. Hist. Surv., **15**(9): 537–550 (reprinted in 1925).

Gerloff, Gerald C., George P. Fitzgerald, and Folke Skoog. 1950. The isolation, purification, and nutrient solution requirements of blue-green algae. IN: Brunel, Prescott, and Tiffany, "The Culturing of Algae," Kettering Foundation, Yellow Springs, Ohio. pp. 27–44.

Gottschall, R. Y., and O. E. Jennings. 1933. Limnological studies at Erie, Pa. Trans. Amer. Micr. Soc., **52**(3): 181–191.

Hardy, Allister C. 1956. The open sea. Its natural history: the world of plankton. Collins, London. pp. i-xv, 1–335, +48 pls.

Hintz, Walter James. 1955. Variations in populations and cell dimensions of phytoplankton in the island region of western Lake Erie. Ohio J. Sci., **55**(5): 271–278.

Hooper, Frank F. 1951. Limnological features of a Minnesota seepage lake. Amer. Midl. Nat., **46**(2): 462–481.

Juday, Chancy. 1942. The summer standing crop of plants and animals in four Wisconsin lakes. Trans. Wis. Acad. Sci. Arts Lett., **34**: 103–135.

Kiefer, Friedrich. 1955. Die Lebensgemeinschaft "Plankton" des Bodensees. Aus der Heimat, **63**(11–12): 201–211.

Kuznetsov, S. I. 1954. [Basic approaches to the study of correlations between the primary production of organic matter and the biogenous mass]. Trudy Problemnykh i Tematicheskikh Soveschanii, Akad. Nauk. S. S. S. R., Problemy Girodbiol. Vnutren. Vod, **2**(2): 202–212 (in Russian).

Lenhardt, L. G. 1955. Water quality and water usage of the Great Lakes public water supplies. IN: Great Lakes Res. Inst. Publ., "The Great Lakes and Michigan" pp. 13–15. Ann Arbor, Mich.

Leverin, Harald A. 1947. Industrial waters of Canada. Report on investigations, 1934 to 1943. Canada Bur. Mines., Rep't. No. **819**: 1–109.

Lindeman, Raymond L. 1942. The trophic-dynamic aspect of ecology. Ecology, **23**(4): 399–418.

MacFadyen, A. 1948. The meaning of productivity in biological systems. J. Anim. Ecol., **17**(1): 75–80.

McMillan, Gladys L., and Jacob Verduin. 1953. Photosynthesis of natural communities dominated by *Cladophora glomerata* and *Ulothrix zonata*. Ohio J. Sci., **53**(6): 373–377.

Meyer, Bernard S., Frank H. Bell, Lawrence C. Thompson, and Edythe I. Clay. 1943. Effect of depth of immersion on apparent photosynthesis in submersed vascular aquatics. Ecology, **24**(3): 393–399.

Naumann, Einar. 1918. Über die natürliche Nahrung des tierischen Limnoplanktons. Ein Beitrag zur Kenntnis des Stoffhaushalts im Süsswasser. Lunds Univ. Årsskrift., N. F. Afd. 2, **14**(31): 1–48.

Ohio Division of Water. 1953. Lake Erie pollution survey. Final report. pp. 1–201, +3 maps. *Ibid.* Supplement. pp. 1–125.

Pennak, Robert W. 1945. Some aspects of the regional limnology of northern Colorado. Univ. Colorado Stud., Ser. D., **2**(2): 263–293.

———. 1946. The dynamics of fresh-water plankton populations. Ecol. Monogr., **16**(4): 340–355.

———. 1949. Annual limnological cycles in some Colorado reservoir lakes. Ecol. Monogr., **19**: 233–267.

———. 1955. Comparative limnology of eight Colorado mountain lakes. Univ. Colorado Stud., Ser. Biol., **2**: 1–75.

Rodhe, Wilhelm. 1948. Environmental requirements of fresh-water plankton algae. Experimental studies in the ecology of phytoplankton. Symbol. Bot. Upsalienses, **10**(1): 1–149.

———. 1955. Can plankton production proceed during winter darkness in subarctic lakes? Proc. Intern. Assoc. Limnol., **12**: 117–122.

Ruttner, Franz. 1952. Grundriss der Limnologie. 2. Auflage. de Gruyter, Berlin. pp. 1–232.

Ruttner, Franz. 1953. Fundamentals of limnology. Univ. Toronto Press. pp. i–xi, 1–242. Translated by D. G. Frey and F. E. J. Fry.

Steeman Nielsen, E. 1937. On the relation be-

tween the quantities of phytoplankton and zooplankton in the sea. Jour. Cons. Perm. Int. Explor. Mer, **12**: 147–154.

Sullivan, C. R. 1953. Survey on the phytoplankton at the mouths of ten Ohio streams entering Lake Erie. In: Ohio Div. of Water, "Lake Erie Pollution Survey. Final Report." pp. 152–156.

Verduin, Jacob. 1951a. A comparison of phytoplankton data obtained by a mobile sampling method with those obtained from a single station. Amer. J. Bot., **38**(1): 5–11.

———. 1951b. Comparison of spring diatom crops of western Lake Erie in 1949 and 1950. Ecology, **32**(4): 662–668.

———. 1954. Phytoplankton and turbidity in western Lake Erie. Ecology, **35**(4): 550–561.

Wagner, Frederick E. 1929. Chemical investigations of the Erie-Niagara watershed. In: State of N. Y. Cons. Dept. "A Biological Survey of the Erie-Niagara Watershed." Suppl. to Ann. Rep't., **18**: 107–133.

Welch, Paul S. 1948. Limnological methods. Blakiston, Philadelphia. pp. i–xviii, 1–381.

Williams, Roger C. 1929. Pollution studies in the light of the chemical analyses. Bull. Buffalo Soc. Nat. Sci., **14**(3): 60–64.

Wright, Stillman, L. H. Tiffany, and Wilbur M. Tidd. 1955. Limnological survey of western Lake Erie. U. S. Fish and Wildl. Serv., Spec. Sci. Rept.: Fish No. **139**: 1–341.

The Rate of Development of *Balanus balanoides* (L.) Larvae[1]

H. Barnes and Margaret Barnes

The Marine Station, Millport, Scotland, and the Woods Hole Oceanographic Institution

ABSTRACT

Experiments are described on the rate of transition of State I to State II *B. balanoides* larvae at a series of temperatures. A value of 2.2 was found for Q_{10} between 1° and 18° C. The data regarding the length of the planktonic phase over the area of distribution are reviewed and new data also given. There is little indication of a high correlation with temperature. The discrepancy may be accounted for by acclimation, but it is suggested that the quality and quantity of food should be considered as interacting with and perhaps compensating for the effect of temperature.

Balanus balanoides (L.) is widely distributed over the arctic-boreal regions; the conditions of its environment, therefore, differ widely, and information regarding rate processes at any stage of the life cycle is clearly of importance in any interpretation of the ecology of the species. Plankton collections at a number of places have given some information regarding the rate of development of the free living larval stages, but there is no recorded experimental work. While such work on the late larval stages involves all the difficulties associated with the culture and maintenance of marine planktonic organisms, the Stage I to Stage II transition may be easily studied. Ripe embryos, teased out from the egg lamellae present in the mantle cavity of the adult readily hatch into Stage I nauplii and soon pass into Stage II nauplii.

MATERIAL AND METHODS

A number of lamellae of the same apparent degree of ripeness were teased out into sea water contained in a petri dish; the liberated Stage I nauplii accumulate on the lighter side of the dish and may be easily pipetted off. Since the rate of transition from Stage I to II changes during the season (under constant experimental conditions) presumably according to the ripeness of the lamellae, all the following experiments were run on a single batch of nauplii after the techniques had been tested in a series of preliminary experiments.

Small samples of active nauplii were pipetted into large test tubes containing 5 ml sea water, brought to and subsequently maintained at constant temperature in water baths; experiments were run at 1.0, 5.5, 6.1, and 18.5°C. Some 40–50 such tubes were set up at each temperature. All the tubes were held in the multiple arms of a single mechanical shaker and gently agitated throughout the experiments, since the preliminary experiments had shown that without gentle shaking the rate of transition is reduced and the results are very erratic. This may be due to the fact that without agitation the nauplii were often crowded together and tended to be less active in the relatively small volume of water used. Whether the lower rate of transition in the absence of gentle agitation is the result of less activity *per se* or whether it is perhaps due to the greater ease of ecdysis among actively swimming animals is unknown. From time to time formalin in the usual strength was added to three replicate tubes at each temperature, the appropriate intervals being selected on the basis of earlier experiments. The number of Stage I and Stage II nauplii were subsequently counted

[1] Contribution No. 928 from the Woods Hole Oceanographic Institution. One of us (H. B.) wishes to acknowledge the receipt of a Research Fellowship from the Institution and leave of absence granted by the Council of the Scottish Marine Biological Association. We wish to thank Dr. B. H. Ketchum for his advice, Mr. H. Turner, Jr., for facilities during the experiments, and Mr. D. Bumpus for information regarding temperatures.

under a low power binocular microscope. From the results the mean percentage of larvae that had moulted to Stage II at any time (corrected for viability by expressing as percentage of ultimate numbers of Stage II) was plotted against the time, and from the smoothed curves the rate of transition, that is, time of half change, was calculated.

RESULTS

These are shown in Figure 1 in which the reciprocal of the time of half change (relative rate of transition) is plotted on a logarithmic scale against temperature. The two are linearly related over the whole range with a Q_{10} of about 2.2. Such a value might have been anticipated for an arctic-boreal species at the range of temperatures of the normal habitat, namely 0–8°C for the planktonic phase (see Scholander *et al.* 1953). However, it is a little surprising that no falling off in the value of Q_{10} is apparent at the higher temperatures—higher than those at which the larvae are normally found but not higher than those to which the adult is subjected in the southern parts of its distribution. This may indicate that the larvae are completely viable up to 18°C and that up to this limit temperature effects on the larval stage do not limit the distribution of the species. However, the Stage I to II transition takes place rapidly; the later stages which take much longer in development may not be viable at the higher temperatures.

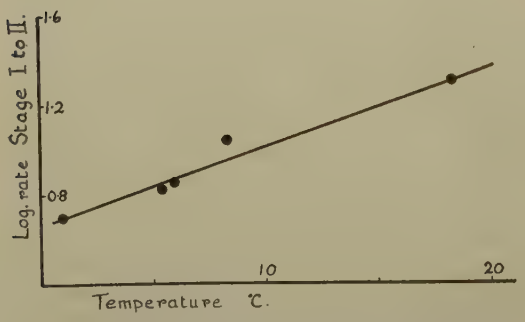

FIG. 1. The effect of temperature on the Stage I to II transition. Animals were kept in filtered sea water and gently agitated.

COMPARISON WITH PLANKTONIC DATA

The results may be compared with what is known of the length of planktonic life under natural conditions. When sampling problems are taken into account, even with collections taken at short intervals, it is difficult to estimate with any accuracy the length of individual stages—usually a matter of only a few days. We may consider the planktonic life up to the cypris; the cypris stage may be variable and estimates based on the settlement dates are even more crude.

There is satisfactory data from the Firth of Clyde (Scotland). Pyefinch (1948) making hauls every three days during the spring months estimated the time for development up to the cypris stage was 32 days in 1944 and 30 days in 1947. We have confirmed these results taking daily hauls with the Hardy plankton indicator; the estimates are the same whether first appearances or peak values are used. The mean sea-surface temperature for this region during the month of March when the larval population is developing is about 7°C (Barnes 1955). Although there is a considerable amount of data on the general biology of *B. balanoides* both round the coast of Britain and Northern France, there are no further reports on plankton collections which allow satisfactory estimates of the larval life to be made.

Again, although there is much literature on the plankton of the Norwegian coast, little attention has so far been paid to the cirripede larvae. However, through the courtesy of Dr. F. K. Wiborg, we have been able to examine plankton hauls taken in the Lofoten area. For the present purpose the hauls were taken too infrequently, but the best estimate using the results from all the stations is some 27–30 days at a temperature of 3–4°C. Runnström (1924–25) while stressing that there may be some variability from year to year gives a plankton life of 30 days for the waters near Bergen at a temperature of 4°C. He also states that in Liverpool Harbour nauplii appeared on February 22 and cyprids on April 6, a period of 43 days.

TABLE 1. *Rate of development of planktonic phase of* B. balanoides

Place	Days	Sea surface temperature °C
Ungava Bay (Canada)	48	1
Halifax Harbour (Canada)	40	1–2
Woods Hole (U. S. A.)	21	6.6
Newport (Rhode Is., U. S. A.)	33	1.0
Millport (Scotland)	32	7.0
Bergen (Norway)	30	4.3
Lofoten (Norway)	27–30	3.0

Bousfield (1952–53) has given much information on *B. balanoides* around the Canadian coast, but the plankton data cover only Halifax Harbour. Weekly samples during the spring of 1952 indicated the first appearance of nauplii on February 26 and Stage VI on April 4; cyprids were settling on the shore by April 10, so that a period of 40 days may be taken as the best estimate for Stage I–VI. During this period ice was observed in the harbor, and Bousfield states that the surface temperatures were less than 3°C. Data by Banks (1955) for Halifax Inner Harbour indicates that 1–2°C is a better estimate for the temperature, a value substantiated by those for Eastport, Maine (U. S. Coast and Geodetic Survey 1955).

We have been able, through the courtesy of Dr. M. J. Dunbar, to examine plankton hauls taken by the Eastern Canadian Arctic Investigations in the Ungava Bay Region. In 1949, Stage II nauplii were first taken on June 4 and cyprids on August 11, which is a period of 48 days for development.

Fish (1925) found in plankton collected at Woods Hole that nauplii first appeared during December and cyprids in February. However, we have recently examined biweekly collections taken by Dr. C. S. Rao at Woods Hole, and while confirming the occurrence of nauplii on December 6 found they were followed by cyprids 21 days later. The mean sea surface temperature at this time was 6.6°C. It would seem that due to conditions pertaining at the time of Fish's collections his nauplii did not give rise to viable cyprids (see Barnes 1956). Fish also records that he observed large quantities of early stage nauplii on January 30 somewhat further to the south at Newport (Rhode Island), and that these were followed by abundant cyprids on March 4, 33 days later; the temperature may be taken as similar to that at Woods Hole for February, namely 1.0°C (U. S. Coast and Geodetic Survey 1955). The data discussed above are collected together in Table 1.

DISCUSSION

It is evident from Table 1 that while at some of the colder places the period of larval development is somewhat longer, there is no regular relation with temperature. In most cases a period of about 30 days is required whatever the temperature. The results stand, therefore, in marked contrast to the effect of temperature on the Stage I to II transition as determined experimentally. It is realised that there is some doubt about feeding in Stage I and that the absolute rates of the different stages may vary; one might expect nevertheless the value of Q_{10} to be similar for all stages. It is possible that acclimation is responsible for the roughly constant period of development, but in this connection it must be noted that the temperature during the later stages of embryonic development varies widely; for example the adults have been buried under the ice in the Canadian Arctic, while at Woods Hole the temperature falls only from 15° in October to 11°C in November. Acclimation to habitat conditions has been shown to be a common feature among poikilothermous animals (Bullock 1955) and has been suggested for a number of planktotrophic larvae by Thorson (1946); the comparisons are, however, largely interspecific.

On the other hand the availability of the right kind of food has been shown to be of fundamental importance in the development of planktonic larvae with high metabolic rates, and it is suggested that this factor may interact with and compensate for temperature effects.

REFERENCES

BANKS, R. E. 1955. Seasonal changes in the water temperature of Halifax Harbour and Bedford Basin. Naval Research Department, Nova Scotia, U/P Section IV, Research Note 55/1.

BARNES, H. 1955. Climatological and salinity data for Millport, Scotland. Glasgow Nat., **17:** 193–204.

———. 1956. *Balanus balanoides* (L.) in the Firth of Clyde: the development and annual variation of the larval population, and the causative factors. J. Anim. Ecol., **25:** 72–84.

BOUSFIELD, E. L. 1952–3. The distribution and spawning seasons of barnacles on the Atlantic coast of Canada. Ann. Rept. Nat. Mus. Canad. 1952–3, Bull. **132:** 112–154.

BULLOCK, T. H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Rev., **30:** 311–342.

FISH, D. J. 1925. Seasonal distribution of the plankton of the Woods Hole region. Bull. U. S. Bur. Fish., **41:** 91–179.

PYEFINCH, K. A. 1948. Notes on the biology of cirripeds. J. Mar. Biol. Ass. U. K., **27:** 464–503.

RUNNSTRÖM, S. 1924–25. On the biology and development of *Balanus balanoides* (Linné). Bergen Mus. Aarbok, Nr. 5, 1–46.

SCHOLANDER, P. F., W. FLAGG, V. WALTERS, AND L. IRVING. 1953. Climatic adaptation in arctic and tropical poikilotherms. Physiol. Zoöl., **26:** 67–92.

THORSON, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates. Medd. Komm. for Havundersøg., Kbh., serie Plankton, **4:** 1–523.

U. S. COAST AND GEODETIC SURVEY. 1955. Surface water temperatures at tide stations. Atlantic Coast, North and South America. U. S. C. & G. S., Spec. Publ. No. 278. 5th Edition.

Some Biochemical Aspects of Red Tides and Related Oceanographic Problems[1]

ALBERT COLLIER

U. S. Fish and Wildlife Service, Galveston, Texas

ABSTRACT

"Red Tides" are blooms of plankton organisms which may or may not be lethal to aquatic organisms. The sudden increase in multiplication rates of the organisms is caused by a complex of biological factors, and physical factors play a major role in effecting mechanical concentration.

This paper deals with the biological factors operative in the genesis of blooms. Nutritional studies show that organic factors which occur naturally in the water are important. The existence of biologically active organic compounds and possible modes of action are discussed. The method of measurement of "carbohydrates" is explained and their production by bacteria-free cultures of *Prorocentrum* is demonstrated. Such organic compounds could serve as substrates for vitamin B_{12}-producing bacteria and thus play a role in the conditioning of water for succeeding populations.

Gymnodinium breve, the causative organism of the Florida Red Tide, responds differentially to Gulf of Mexico waters of different origins. A suggested explanation is the interaction between environmental copper and hydrogen sulfide. Once started, a bloom may replenish the required nutrients by the decomposition of the fish that it kills.

INTRODUCTION

"Red Tide" is a popular term applied to unusual concentrations of freshwater or marine organisms—protozoans or unicellular algae—which may or may not cause a red discoloration of the water, and which may or may not be lethal to fish or other aquatic organisms. In recent years the phenomenon has become widely known and the term much used because of some highly publicized and spectacular fish kills. The explanation of the genesis of these blooms is to be found in the physiology and related environmental responses of the organisms concerned, while the extent of resulting fish kills is dependent upon their concentration. Biological and chemical factors are most critical in determining the environmental responses of the causative organisms, while physical factors are the most important concentrating agents.

The mechanics of concentration of semibuoyant particles drifting in the open sea can be explained by such devices as convergence of water masses, wind-driven surface currents, and convection cells. Various authors have treated these systems in theoretical terms, and Ryther (1955) has reviewed them in the light of the present knowledge of dinoflagellate nutrition. He concluded, "It is necessary only to have conditions favoring growth and dominance of a moderately large population of a given species and the proper hydrographic and meteorological conditions to permit the accumulation of organisms at the surface and to effect their further concentrations in localized areas."

The U. S. Fish and Wildlife Service has concentrated on the study of the biological processes that stimulate plankton blooms, and the collection of field data necessary for the interpretation of laboratory results. The following is a review of some of our more recent findings of general interest and a discussion of the relations between the natural organic compounds in sea water and plankton blooms. Specific oceanographic aspects will be emphasized.

I wish to acknowledge the work of my colleagues, Mr. J. O. Bell, Mr. Alexander Dragovich, Mr. K. T. Marvin, Dr. Sammy Ray, Dr. T. J. Starr, Mr. W. B. Wilson, and Mrs. Zoula Zein-Eldin, who have made

[1] The substance of this paper was contained in an address before the Symposium on Marine Biochemistry, 131st Meeting of the American Chemical Society, Miami, Florida, April 9, 1957.

contributions to the information used in this paper.

NUTRITIONAL STUDIES ON DINOFLAGELLATES

Although we are studying the nutrition of dinoflagellates in general, our initial attempts concerned the isolation and continued culture of *Gymnodinium breve*, the causative organism of the Florida Red Tide (Wilson and Collier 1955, Ray and Wilson 1957). The composition of the most useful medium, and one which we are still using, is given in Table 1.

With this as a starting point we progressed from unialgal cultures to bacteria-free cultures (Ray and Wilson 1957) and have worked intensively to derive a defined medium. Many workers have put much effort into this problem, using a variety of organisms (Pringsheim 1946, Barker 1935, Hutner and Provasoli 1955, and Provasoli and Pintner 1953). Optimum limits for inorganic salts, trace metals, vitamins, amino acids, and miscellaneous growth factors have been determined empirically. Even with all this information and the results of our own work we are still left with the conclusion that some sea waters contain organic materials that make the addition of soil extract or vitamin B_{12} unnecessary.

Thus we became concerned with the biological controls which systems of aquatic organisms can exert on each other through the intermediary of organic substances. Through metabolites exuded by living cells, or decomposition products from dead cells, a population may exclude other populations, or promote or inhibit the growth of succeeding populations (Stevens 1949, Wilson 1951, Lucas 1949, and Collier 1953).

TABLE 1. *Medium for the unialgal isolation of* G. breve *Davis*

After all additions are made, there is approximately 110 ml of medium.
(After Wilson and Collier 1955)

Aged sea water (salinity, about 36.5 ‰)	95.0 ml
Distilled water*	5.0 ml
NH_4Cl†	0.1 mg
KH_2PO_4†	0.05 mg
$MgCl_2 \cdot 6H_2O$†	0.02 mg
$Na_2S \cdot 9H_2O$†	0.1 mg
$NaHCO_3$†	0.1 mg
Vitamin B_{12}	0.1 μg
Thiamine hydrochloride	1.0 mg
Biotin	0.05 μg
Soil extract‡	2.0 ml
EDTA·Na (6 ml of 0.25-percent solution)	15.0 mg

* More or less may be required, depending on the salinity.

† Added as 0.5 ml of the following solution of the components of van Niel's medium for sulfur bacteria: NH_4Cl, 0.2 g; KH_2PO_4, 0.1 g; $MgCl_2 \cdot 6H_2O$, 0.04 g; $NaHCO_3$, 0.1 g; and $Na_2S \cdot 9H_2O$, 0.2 g to 1 liter of distilled water.

‡ Simmer for 40 min a mixture of 500 g of garden soil and 1 liter of distilled water. Let it stand for 4 days and decant the supernatant. Repeat simmering and decantation until extract is clear. Use the supernatant.

THE DEMONSTRATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN SEA WATER

Collier *et al.* (1953), using a modified Dische reaction (Erdman and Little 1950), reported carbohydrate-like materials which quantitatively affected the pumping rate of oysters, and whose concentration in untreated water was influenced by light. They varied in concentrations from zero to over 20 milligrams per liter in the estuary studied. Wangersky (1952) reported the identification of dehydro-ascorbic acid and a rhamnoside from natural sea water. High concentrations of carbohydrates were found in rich marsh waters and in extreme concentrations of plankton organisms associated with a red tide outbreak in Florida (Collier 1953). These latter values were on the order of 50 mg per liter.

Interesting results of the study of the specific effects of commercially prepared organic compounds on various organisms were the motor responses elicited in oysters and barnacles by niacinamide and ascorbic acid, respectively (Collier *et al.* 1956). The effective concentrations were 0.042 mg per liter of sea water for niacinamide and 0.014 mg per liter for ascorbic acid. This is a further demonstration of the potential role of organic compounds in the marine community.

THE MEASUREMENT OF "CARBOHYDRATES" IN NATURAL SEA WATER

Before discussing the distribution of organic substances in the Gulf of Mexico and their production in laboratory cultures some analytical details should be clarified. The use of N-ethyl carbazole for the quantitative estimation of carbohydrates in marine waters was originally described by Erdman and Little (1950) and has been improved by Zein-Eldin and May (1957). Some time ago we found that in the waters with which we were working anthrone did not provide the sensitivity of N-ethyl carbazole, and as a result we adopted the latter as our standard reagent.

The analytical results are reported in terms of "milligrams per liter (arabinose equivalent)," but will be referred to hereafter as simply "carbohydrates." The reason for this is that we do not know what specific compounds are being measured, but only that on the hydrolysis of a sample we get a color response which can be quantitated according to Beer's Law. Zein-Eldin has plotted a series of absorption curves for a variety of hexoses and pentoses, and she kindly provided me with the figures used here to compare hexose and pentose curves with those obtained from naturally produced substances.

The absorption curve for a bacteria-free culture of *Prorocentrum* sp. is compared with the curves for arabinose and sucrose in Figure 1. The original traces were made with a recording spectrophotometer. The curve made from the culture clearly indicates a mixture of pentoses and hexoses.

The origin, significance, and molecular structures of these compounds raise some questions which need to be answered. Lewis and Rakestraw (1955) found that filtered samples of sea water do not yield significant quantities of carbohydrate in the Pacific. On the other hand, Plunkett and Rakestraw (1955) show heterogeneous distribution of dissolved organic materials in the Pacific as determined by Krogh's method for organic carbon. They considered these differences significant and concluded that since these materials represent the largest fraction of total organic matter in the sea, more intensive investigation is needed. Kay (1954), using the determination of elemental organic carbon, found a situation in the Kielerbucht similar to that reported by Plunkett and Rakestraw. After discussing his findings and pointing out conflicting arguments, he concluded that the whole matter must await clarification of the exudation of organic metabolites by the plankton organisms, particularly the algae. He considered the latter to be a plausible source of dissolved organic compounds in the sea. Vallentyne (1957) has given a comprehensive review and classification of organic compounds reported in the literature.

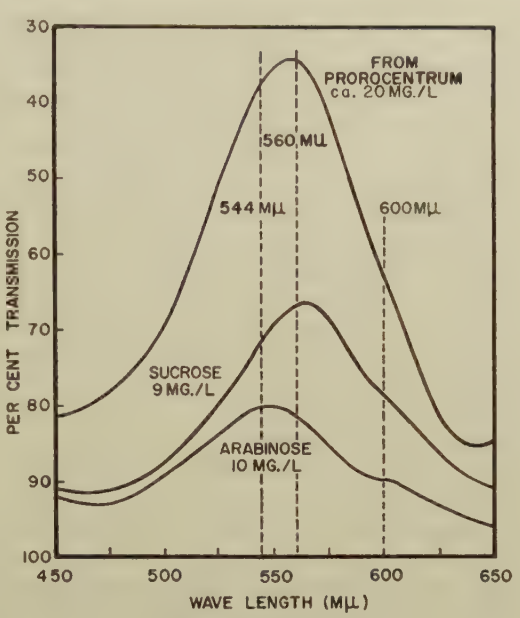

FIG. 1. Photometric responses of sucrose, arabinose, and culture of *Prorocentrum* sp. after treatment with N-ethyl carbazole. Concentrations are indicated on curves. Traces were made with recording spectrophotometer.

PRODUCTION OF ORGANIC SUBSTANCE IN LABORATORY CULTURES

In the preceding section I offered evidence that the carbohydrate responding to N-ethyl carbazole and produced by a single, specific organism in bacteria-free culture could be qualitatively classed with known sugars.

To understand the significance of measurements of these compounds made on samples from natural environments we should have some idea of the quantitative aspect.

For this purpose I present some results from studies on a 16-liter, unialgal (not bacteria-free) culture of *Prorocentrum* sp. which contained approximately 2.8 million cells per liter. The organisms were separated from the medium with a high-speed continuous-flow centrifuge. Although bacteria were present, it is doubtful that they contributed greatly to the carbohydrate values when *Prorocentrum* was by far the dominant organism. This situation is similar to that found by Goldberg *et al.* (1951) in their study on phosphate utilization by diatoms. Five independent carbohydrate determinations were made for the centrifugate, the residue, and the basal medium. The means of these determinations and their respective standard deviations are given here. A mean value of 0.72 ± .22 mg/L is given for the basal medium. This initial quantity is not subtracted from the final results because of the likelihood that it served as a carbon source for the organisms in the culture and was redistributed between them and their metabolites. The mean carbohydrate value for the whole culture was 9.58 ± .48 mg/L, and for the centrifugate 5.84 ± .44 mg/L. The mean difference of 3.74 ± .46 mg/L represented the portion of the material retained by those organisms not passing through the centrifuge. The total carbohydrate in the centrifugate was about 60% of that in the original culture, and estimating 10% of this as originating in crushed organisms or those not retained in the residue, this culture produced approximately 2 mg of non-particulate carbohydrate per million cells. In terms of the whole culture the production was 3.4 mg of carbohydrate per million cells. Similar experiments with *G. breve* and *Gymnodinium splendens* gave values of 2 mg and 1.6 mg, respectively, for the centrifugates. We cannot rely on either of the latter because the organisms were too fragile for an evaluation of their resistance to the mechanical effects of centrifuging. *Prorocentrum* is an armoured dinoflagellate and is more resistant than the naked forms mentioned above.

Lewin (1956) found that *Chlamydomonas mexicana* nom. prov., a mucilaginous freshwater flagellate, liberated as much as 0.1 gram per liter of soluble polysaccharide into the culture medium. This material constituted 20–25% of the total organic matter produced. While these values cannot be compared to our results from the *Prorocentrum* cultures, they indicate the magnitude of the potential role of algae in producing free organic substances in the aquatic environment.

THE DISTRIBUTION OF CARBOHYDRATES IN THE COASTAL AND ESTUARIAL WATERS OF THE GULF OF MEXICO

In earlier work Collier *et al.* (1953) found that carbohydrates in marsh waters fluctuated between zero and 25 mg/L. Such a range should have been expected in an area which was influenced both by the carbohydrate-poor waters of the open Gulf of Mexico and some highly productive marsh areas. The same work also showed a clear diurnal cycle of concentrations, with the maximum coming at midafternoon and the minimum after midnight. In samples taken from the high concentrations of *G. breve* in a red tide I reported (1953) some 50 mg of crystalline material per liter of water. This is not out of line with 44 mg/L of carbohydrates measured in a 16-liter unialgal (not bacteria-free) culture of *Prorocentrum*. Our evidence supports the idea that, in surface waters at least, some of these substances are produced by photosynthetic organisms, and when such organisms are in sufficiently dense aggregations, significant quantities of these substances will be indicated by the N-ethyl carbazole reaction.

The patchiness or highly uneven distribution of organisms in marine waters is well established (Barnes and Marshall 1951, Bainbridge 1953), and if the carbohydrates are associated with such aggregates of photosynthetic organisms we should find them similarly distributed.

In a series of oceanographic cruises in the Gulf of Mexico by the U. S. Fish and Wildlife Service research vessel ALASKA samples

for the determination of carbohydrates, tyrosine, and organic phosphorus were collected at depths ranging from the surface to 2500 meters at stations distributed over the entire Gulf. There were over 800 determinations each of carbohydrate, tyrosine, and organic phosphorus in this series. A detailed study of this investigation will appear elsewhere, but it is helpful to present some of this information here to indicate how consistent are the results of organic analyses with heterogeneous plankton distribution.

On one of the cruises ALASKA encountered an area of steep salinity gradient that was indicative of a convergence of two dissimilar water masses. In the area there was "red water" caused by a heavy concentration of a ciliate, probably *Mesodinium pulex* (Slobodkin 1953). This bloom was well offshore and bore no resemblance to a fish-killing red tide such as is caused by *G. breve.* The concentration of the organisms is consistent with the mechanisms surveyed by Ryther (1955). In samples of water collected from this station (preserved by quick freezing and neither filtered nor centrifuged) we got the following values: carbohydrates 3.6 mg/L, tyrosine 1.4 mg/L, and organic phosphorus 6.7 microgram-atoms PO_4-P/L. At a station in clear water outside of the ciliate concentration we obtained the following values: carbohydrates 0.0 mg/L, tyrosine 0.3 mg/L, and organic phosphorus 1.0 microgram-atoms PO_4-P/L. These differences demonstrate the association of high carbohydrates, tyrosine, and organic phosphorus values with high concentration of organisms.

Table 2 illustrates the frequency distribution of carbohydrates, tyrosine, and organic phosphorus in all samples taken within the 100-fathom curve by M/V ALASKA. By inspection one can see that these distributions are similar to those shown by Barnes and Marshall (1951) to characterize plankton aggregations. This similarity may be interpreted to mean that the concentration of organic substances represented by the carbohydrate, tyrosine, and organic phosphorus tests were generally low, but when they did occur the concentrations were relatively high, or, in other words, there was a tendency towards the contagious distribution (Barnes and Marshall 1951).

TABLE 2. *Frequency distributions of carbohydrates, tyrosine, and organic phosphorus for samples collected over continental shelf of Gulf of Mexico by M/V Alaska*

Carbohydrates			Tyrosine			Organic Phosphorus		
mg/L	*n*	%	mg/L	*n*	%	μg-at. PO_4-P/L	*n*	%
.0	68	23.2	.0	63	19.7	.0	13	6.7
.2	37	12.6	.2	73	22.8	.2	35	18.1
.4	41	14.0	.4	63	19.7	.4	34	17.6
.6	32	11.0	.6	41	12.8	.6	32	16.6
.8	24	8.2	.8	32	10.0	.8	31	16.1
1.0	22	7.5	1.0	11	3.4	1.0	15	7.8
1.2	20	6.8	1.2	16	5.0	1.2	11	5.7
1.4	10	3.4	1.4	10	3.1	1.4	3	1.6
1.6	14	4.8	1.6	3	0.9	1.6	0	—
1.8	8	2.7	1.8	6	1.9	1 8	4	2.1
2.0	7	2.4	2.0	2	0.6	2.0	4	2.1
2.2	1	0.3	—	—	—	2.2	5	2.6
2.4	2	0.7	—	—	—	2.4	2	1.0
2.6	3	1.0	—	—	—	2.6	2	1.0
2.8	2	0.7	—	—	—	2.8	0	—
3.0	2	0.7	—	—	—	3.0	2	1.0
	293	100.0		320	99.9		193	100.0

ORGANIC COMPOUNDS AND RED TIDES

We may now deal more specifically with the Florida Red Tide. Over a period of several months a series of stations along the Florida coast has been sampled for carbohydrates, proteins, and other chemical entities. These data will receive a more detailed treatment elsewhere, but I would like to mention some of the outstanding features of the analysis in terms of the genesis of red tides.

There were 184 pairs of protein and carbohydrate determinations used in this study. The extremes of carbohydrate values were 0.0 and 19.4 mg per liter; of tyrosine, 0.0 and 2.9 mg per liter. The highest concentrations in both cases were found in rivers, tidal streams, and marshes bordering the estuaries; the concentration generally diminished in a seaward direction, with the lowest values occurring in the offshore waters of the Gulf. The latter were within the general range of the quantities reported from the ALASKA cruises.

At this point the most important feature is the erratic nature of the distributions of

these two classes of compounds and, as pointed out above, the probability that they are directly related to the distribution of microplankton.

Organic compounds provide a substrate for bacterial activity and can serve as growth promoters for animal and plant components of the plankton. A bloom of algae can charge the water with sufficient organic material to support a growth of bacteria and/or to condition the water for succeeding species. We have found that a particular bacterial colony type always associated with our unialgal cultures of *G. breve* as the dominant bacterium is a producer of vitamin B_{12}, a growth promoter. This bacterium will not develop to a position of dominance in the absence of *G. breve*. Here, then, is at least an experimental system in which the alga is probably supplying an organic substrate which indirectly conditions the water for its own growth. In combination with the distribution of organic compounds detailed above, this observation leads to some experiments similar to those conducted by Wilson (1951). The following is the summary of his paper: "Eggs and larvae of *Echinus esculentus, Ophelia bicornis* and *Sabellaria alveolata* developed abnormally, or were in poor health, in seawater collected from the English Channel in the region of the Eddystone, although in water collected from the Celtic Sea development was generally normal and healthy. Experiments indicated that the Channel water lacked some unknown constituent, essential for healthy development of these species, present in the Celtic Sea. The results accord with some of the hydrographical and biological changes in the area which have taken place within recent years."

Mr. W. B. Wilson of our laboratory at Galveston has been conducting growth experiments with cultures of *G. breve* using sea water collected at different times from various points as a base for his culture media. At one extreme some of these waters are outright toxic to the organisms, and at the other the waters will support luxuriant growth with no supplementary additions. There are intermediate types which cause response after treatment with additives ranging from a single item of the above formula (Table 1) to the whole list. The extremes may come from samples collected at the same time only a few miles apart.

We have found that sulfides will substitute for organic chelators and that copper is highly toxic to *G. breve*. Sulfides were used in the original medium for the artificial culture of *G. breve* (Table 1). The mangrove-covered marshes that border the estuaries of the Florida west coast are prolific producers of hydrogen sulfide, and the natural copper concentration in the coastal waters is extremely variable. The interplay of these two factors is possibly active in establishing the level of toxicity of untreated sea water to this and other organisms. An additional factor may be the action of natural organic compounds as chelators, because they themselves can act as copper binders. Thus it may be possible for them simultaneously to detoxify water and to furnish growth promoters for the more sensitive members of the marine plankton.

A constant surveillance in Florida waters over the past three years has shown that *G. breve* is present in very low concentrations at one point or another along the Florida coast most of the time. Mr. W. B. Wilson has successfully isolated and cultured the organism from waters within the city limits of Galveston, Texas. Wilson and Ray (1956) found blooms of the same organism associated with large fish kills off the South Texas and Mexican east coasts, and again it was successfully isolated and cultured. From these findings we conclude that the organism may normally exist over a considerable geographic range in very low numbers.

Its sensitivity to unfavorable conditions keeps it in check and prevents a more frequent occurrence of Red Tides. However during the rainy season the introduction of large volumes of land drainage with heavy loads of organic materials can provide the initial stimulus for heavy blooms of the organism, which in turn may augment themselves with both inorganic and organic materials produced by the decomposition products of dead fish (Wilson and Collier 1955).

REFERENCES

BAINBRIDGE, R. 1953. Studies on the interrelationships of zooplankton and phytoplankton. J. Mar. Biol. Assoc. U. K., **32**: 385–447.

BARKER, H. A. 1935. The culture and physiology of marine dinoflagellates. Arch. Mikrobiol., **6**: 157–181.

BARNES, H., AND S. M. MARSHALL. 1951. On the variability of replicate plankton samples and some applications of "contagious" series to the statistical distribution of catches over restricted periods. J. Mar. Biol. Assoc. U. K., **30**: 233–263.

COLLIER, A. 1953. The significance of organic compounds in sea water. Trans. No. Amer. Wildl. Conf., March 1953, **18**: 463–472.

COLLIER, A., S. M. RAY, A. W. MAGNITZKY, AND J. O. BELL. 1953. Effect of dissolved organic substances on oysters. U. S. Dept. Int., Fish and Wildlife Service, Fish. Bull., **84**: 167–185.

COLLIER, A., S. M. RAY, AND W. B. WILSON. 1956. Some effects of specific organic compounds on marine organisms. Science, **124**: 220.

ERDMAN, J. G., AND A. B. LITTLE. 1950. Special report on the analysis of marine and coastal and estuarial waters. Mellon Inst. Indus. Res., Univ. Pittsburgh. May 1950.

GOLDBERG, E. D., T. J. WALKER, AND A. WHISENAND. 1951. Phosphate utilization by diatoms. Biol. Bull., **101**(3): 274–284.

HUTNER, S. H., AND L. PROVASOLI. 1955. Comparative biochemistry of flagellates. pp. 17–43. In: Biochemistry and Physiology of Protozoa, Vol. II, Ed. S. H. Hutner and André Lwoff. Acad. Press, Inc., New York. pp. i–xiii, 14–388.

KAY, HANS. 1954. Untersuchungen zur Menge und Verteilung der Organischen Substanz im Meerwasser. Kieler Meeresforsch., **10**: 202–214.

LEWIN, R. A. 1956. Extracellular polysaccharides of green algae. Can. J. Microbiol., **2**: 665–672.

LEWIS, G. J. JR., AND N. W. RAKESTRAW. 1955. Carbohydrate in sea water. J. Mar. Res., **14**: 253–258.

LUCAS, C. E. 1949. External metabolites and ecological adaptation. Symp. Soc. Exp. Biol. No. III, pp. 336–356.

PLUNKETT, M. A., AND N. W. RAKESTRAW. 1955. Dissolved organic matter in the sea. Deep-Sea Res. Suppl., **3**: 12–14.

PRINGSHEIM, E. G. 1946. Pure cultures of algae. Cambridge Univ. Press. pp. i–xii, 13–119.

PROVASOLI, L., AND I. J. PINTNER. 1953. Ecological implications of *in vitro* nutritional requirements of algal flagellates. Ann. N. Y. Acad. Sci., **56**: 839–851.

RAY, S. M., AND W. B. WILSON. 1957. The effects of unialgal and bacteria-free cultures of *Gymnodinium breve* on fish and notes on related studies with bacteria. U. S. Dept. Int., Fish and Wildlife Service, Fish. Bull. In press.

RYTHER, J. H. 1955. Ecology of autotrophic marine dinoflagellates with reference to red water conditions. pp. 387–414. In: The Luminescence of Biological Systems. Ed. Frank H. Johnson. AAAS, Wash., D. C. pp. i–xiv, 15–452.

SLOBODKIN, L. B. 1953. A possible initial condition for red tides on the coast of Florida. J. Mar. Res., **12**: 148–155.

STEVENS, G. A. 1949. Contributions to the biology of the mackerel *Scomber scombrus* L. II. A study of the fishery in the southwest of England, with special reference to spawning, feeding, and "Fishermen's signs." J. Mar. Biol. Assoc. U. K., **28**: 555–576.

VALLENTYNE, J. R. 1957. The molecular nature of organic matter in lakes and oceans, with lesser reference to sewage and terrestrial soils. J. Fish. Res. Bd. Canada, **14**(1): 33–82.

WANGERSKY, P. J. 1952. Isolation of ascorbic acid and rhamnosides from sea water. Science, **115**: 685.

WILSON, D. P. 1951. A biological difference between natural sea waters. J. Mar. Biol. Assoc. U. K., **30**: 1–20.

WILSON, W. B., AND A. COLLIER. 1955. Preliminary notes on the culturing of *Gymnodinium breve* Davis. Science, **121**: 394–395.

WILSON, W. B., AND S. M. RAY. 1956. The occurrence of *Gymnodium breve* in the western Gulf of Mexico. Ecology, **37**(2): 388.

ZEIN-ELDIN, Z. P., AND B. Z. MAY. 1957. An improved method for the determination of carbohydrates with N-ethyl carbazole. (Unpub. manus. on file at U. S. Fish and Wildlife Service, Galveston, Texas).

The Physical Limnology of Northern Britain: an Epitome of the Bathymetrical Survey of the Scottish Freshwater Lochs, 1897–1909[1]

EVILLE GORHAM

Freshwater Biological Association, Ambleside, England

ABSTRACT

Interrelations between drainage area, lake area, length, mean breadth, mean depth, and maximum depth are examined in 262 rock basins and 137 basins lying in or dammed by glacial drift. The effect of lake dimensions upon the time taken for complete water replacement is calculated, and an illustration is given of the influence of lake area and mean depth upon the temperature of surface and bottom waters. No relation is evident between lake dimensions and the organic content (ignition loss) of the profundal muds.

INTRODUCTION

It is almost axiomatic in limnology that the size and shape of a lake are of very great importance in determining its biological characteristics. Nonetheless, apart from a recent paper by Hayes (1957) there does not appear to be much detailed information available concerning the quantitative interrelations between lake dimensions, which must markedly affect many physical properties of lake waters (*i.a.*, temperature, stratification, water movements, rate of replacement), and ultimately the organisms inhabiting them. The present account, which summarizes several years of observations by Sir John Murray, Mr. Laurence Pullar, and their associates (1910; see also the *Geographical Journal* for some years prior to this date), attempts to examine (a) some of the interrelations between drainage area, lake area, length, mean breadth, mean depth, and maximum depth for 399 lakes distributed over the whole of Scotland, of which 262 represent rock basins and 137 are basins lying in or at least dammed by glacial drift; (b) the effect of lake dimensions upon the average time taken for complete water replacement; (c) the seasonal changes of water temperature in lakes of different size; and (d) the possibility of a relation between the organic content of profundal muds and lake dimensions.

[1] The writer is much indebted to Dr. A. C. Stephen for supplying samples of lake muds, and to Prof. S. E. Hollingworth for helpful advice concerning the glacial sculpture of lakes.

INTERRELATIONS BETWEEN LAKE DIMENSIONS

Statistics for length, mean breadth, drainage area, lake area, mean and maximum depth are given in Table 1 for those lakes distinguished as either rock or drift basins by Peach and Horne in the Bathymetrical Survey report of 1910. The average values shown are based upon logarithmic groupings of the data, which bring the frequency distributions much closer to the normal frequency curve than those utilizing untransformed data, and also appear to give reasonably linear regressions. Table 2 presents coefficients of correlation between certain of the transformed dimensions, and Table 3 the corresponding regression formulae.

Length and mean breadth

It might be thought that the length and breadth of a lake are unlikely to be of much limnological significance. However, Mortimer's recent work on temperature seiches (1952, 1953) has shown that the exposure of a lake to wind, which is partly determined by the direction and length of its main axis, has a considerable effect upon water movements, and no doubt through these upon the inhabitants of the lake. Sediment distribution is probably also influenced by such

TABLE 1. *Statistics for Scottish Lakes* (log means ± standard deviations for 262 rock basins and 137 drift basins)

	Rock basins	Drift basins
Length (mi)	$\bar{0}.097 \pm 0.407$	$\bar{1}.818 \pm 0.289$
Mean breadth (mi)	$\bar{1}.290 \pm 0.264$	$\bar{1}.243 \pm 0.265$
Drainage area (mi²)	0.743 ± 0.785	$\bar{0}.348 \pm 0.728$
Lake area (mi²)	$\bar{1}.387 \pm 0.623$	$\bar{1}.066 \pm 0.507$
Mean depth (ft)	1.347 ± 0.404	0.900 ± 0.329
Maximum depth (ft)	1.782 ± 0.405	1.284 ± 0.377

TABLE 2. *Correlation coefficients* (r) *for logarithms of lake dimensions*

	Rock basins	Drift basins
Mean breadth and length	0.75	0.69
Drainage area and lake area	0.70	0.69
Mean depth and lake area[1]	0.68	0.24
Maximum depth and mean depth	0.95	0.92

[1] Combined $r = 0.56$

TABLE 3. *Formulae relating lake dimensions to one another*

y	x	Rock basins	Drift basins
Mean breadth (mi)	Length (mi)	$\log y = 0.483 \log x - 0.757$	$\log y = 0.635 \log x - 0.641$
Drainage area (mi²)	Lake area (mi²)	$\log y = 0.883 \log x + 1.284$	$\log y = 0.984 \log x + 1.267$
Mean depth (ft)	Lake area (mi²)	$\log y = 0.444 \log x + 1.619$	$\log y = 0.153 \log x + 1.043$
Max. depth (ft)	Mean depth (ft)	$\log y = 0.955 \log x + 0.496$	$\log y = 1.060 \log x + 0.330$

water movements (cf. Munk 1941 and Revelle 1939, cited by Mortimer 1952), and it may therefore be worthwhile to examine the horizontal dimensions of lakes to see whether any regularities of pattern emerge.

In Scotland the relationship between length of lake and mean breadth (= area/length) is rather close, the correlation coefficients for the logarithmically transformed dimensions being 0.75 and 0.69 for rock and drift basins, respectively. The regressions of mean breadth upon length are shown on logarithmic scales in Figure 1, where it is evident that there is some difference between the two types of lake basin, the rock basins being narrower than those in or dammed by drift, as well as a good deal larger on the average.

The ratios of length to mean breadth (constructed from the above-mentioned regression lines) are also shown arithmetically in Figure 1; and it is apparent from these that the largest rock basins are exceedingly narrow, since the ratios rise well above 20 in comparison with values of about 3 in the smallest basins of this type. Such a contrast is explained by the situation of the large rock basin lakes in deep mountain valleys, while a great many of the smaller ones lie in plateau basins. Many of the larger drift lakes, with ratios more than treble those of the smallest, also lie in valleys, the barriers being deposits of glacial detritus instead of rock sills, but in general these valleys are rather wide and open, so that the ratios of *length/mean breadth* do not attain high levels.

Drainage area and lake area

The ratio of drainage area to lake area must have considerable importance for hydrobiology, since it will influence the rate of transport of water through a lake. The relation between drainage area and lake area in Scotland is quite close, as shown by the highly significant coefficients of 0.70 and 0.69 for rock and drift basins, respectively. The regressions of the former on the latter are shown in Figure 2, again on logarithmic coordinates, and it may be noted that the difference in slope is significant at the 1 per cent level, though not apparently very great. The *drainage area/lake area* ratio is also plotted arithmetically on Figure 2 in relation to lake area. For rock basins there is a distinct decrease in ratio with increasing lake size. In the smallest lochans of around 0.01 mi² the areas of the drainage basins are in general a little over 30 times those of the lake basins, while for the largest lochs of over 20 mi² the ratio is only about 13. The factor responsible for this difference in ratio cannot be identified with any certainty. However, as a tentative hypothesis it may be suggested that since the larger rock basins are presumably the result of a greater degree of glacial erosion and deepening in the larger mountain valleys than in the tributary val-

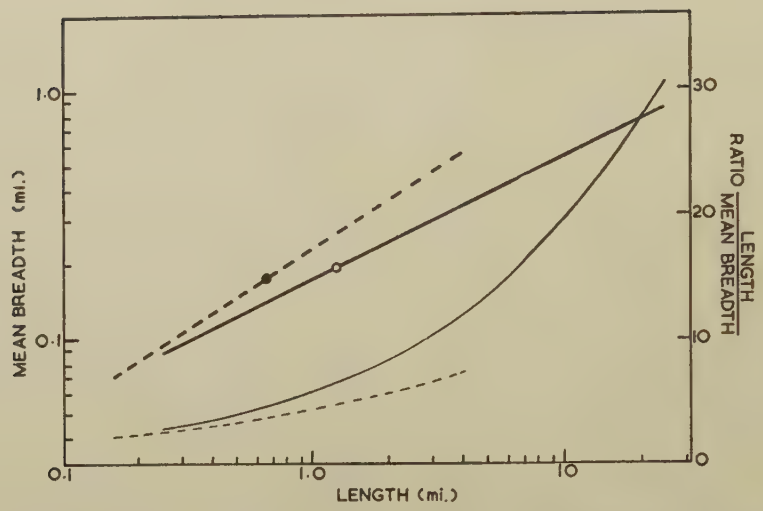

FIG. 1. The relation between length and mean breadth in Scottish lakes. The heavy solid line represents rock basins and the heavy dashed line drift basins, with the circles marking the log means. The lighter lines represent the ratio of length to mean breadth.

leys or on plateaux, the larger lakes might therefore be expected to cover a correspondingly greater proportion of their total drainage area. In the drift basin lakes the ratio is intermediate, the average ranging from about 20 for the smallest to 18 for the largest body of water. Evidently the deposition or subsequent redistribution of the drift has been sufficiently regular to prevent wide variations in this ratio.

Among the drift basins some 29 were segregated by the geologists associated with the lake survey as kettle holes left after the melting of isolated blocks of glacier ice. Since these represent a rather special type of drift basin, separate correlation and regression analyses were carried out, which revealed some difference from other drift basins with regard to the relation between drainage area and lake area. The correlation coefficient between the two dimensions is somewhat higher than usual, 0.74, and the regression of drainage area upon lake area follows the formula $\log y = 1.033 \log x + 1.117$. Thus the ratio of drainage area to lake area is uncommonly low, ranging on the average from about 11 to 13 between the smallest and largest lakes. Such a low ratio is perhaps not unexpected for the rather flat terrain provided by fluvio-glacial deposits, of which these kettle holes are characteristic.

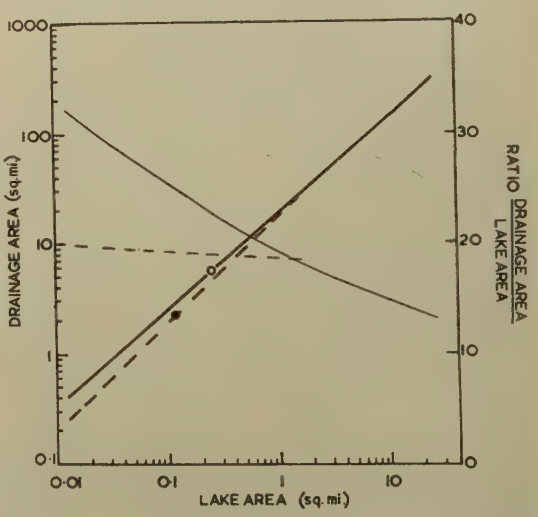

FIG. 2. The relation between drainage area and lake area. The heavy solid line represents rock basins, heavy dashed line drift basins, and circles log means. Ratios are given by lighter lines.

Mean depth and lake area

The mean depth of a lake has been claimed to exert a marked influence upon productivity, especially by Rawson (1955) and more recently by Hayes (1957), and this dimension must certainly be of great importance in determining how much of a lake's

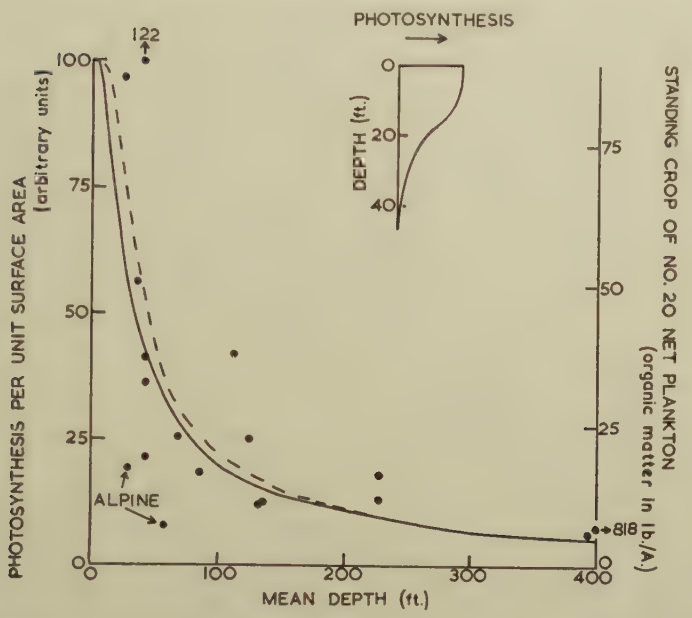

FIG. 3. The influence of mean depth upon areal photosynthesis, under the hypothetical conditions given in the text, and upon the standing crop of no. 20 net plankton in some Canadian lakes. (Solid line represents conical basin, dashed line cylindrical basin; dots give plankton data from Rawson 1953.)

volume lies within the photosynthetic zone. To examine this matter briefly and in a rather crude fashion let us consider all lakes as either cylinders or cones, and as having the photosynthesis/depth relationship shown in the inset in Figure 3. (This curve is taken from Figure 1c from Talling 1957, with the depth scale a rough average of those in his Figures 2–4. The lake is Windermere, about 6 mi² in area and with a mean depth of approximately 80 ft.) The influence of mean depth upon photosynthesis per unit surface area is then shown by Figure 3, in which the solid line represents a conical and the dashed line a cylindrical basin. From these curves (in which stratification is neglected) it is apparent that on the basis of the above assumptions we may expect a steep decline in areal photosynthesis as mean depth increases to about 80–100 ft, after which the fall-off with increasing depth will become much more gradual. As a point of interest Rawson's (1953) data for standing crop of net plankton in central Canadian lakes are also plotted on Figure 3, where the observed points appear to follow a curve generally similar to the theoretical ones, suggesting that the influence of mean depth may well be exerted mainly through its limitation of the time spent by primary producers in the photosynthetic zone, although numerous other factors may of course be expected to complicate such a simple relationship.

To return to the Scottish lakes, the correlation between mean depth and lake area is very clear in the case of the rock basins ($r = 0.68$), but much less so in the drift basins ($r = 0.24$), though the coefficient is still significant at the 1 per cent level. The regressions of mean depth on lake area are illustrated in Figure 4, which demonstrates that while the smallest lakes of both types have much the same mean depth, there is a marked divergence in the larger bodies of water, with the drift basin lakes only a little more than one-quarter as deep as rock basin lakes of similar area. Incidentally, the line given by Hayes (1957) for 500 lakes all over the world lies between the two shown in Figure 4.

In order to ascertain how lake depth varies with increasing horizontal dimensions, the ratio of mean depth to the square root of lake area has been calculated, and is also illustrated in Figure 4. It shows a distinct

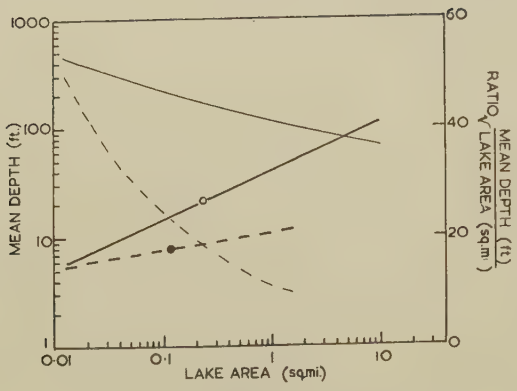

FIG. 4. The relation between mean depth and lake area. The heavy solid line represents rock basins, heavy dashed line drift basins, and circles log means. Ratios are given by lighter lines.

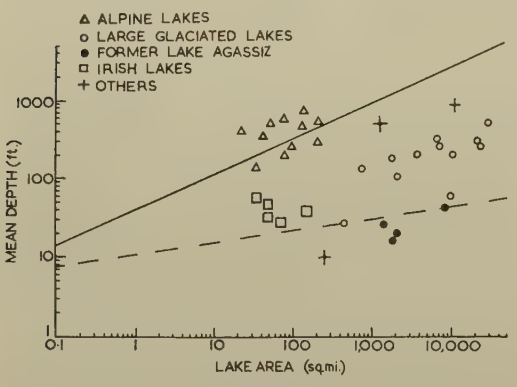

FIG. 5. The relation between mean depth and lake area in some of the largest lakes of the world. The solid and dashed lines represent extrapolations of the lines for Scottish rock and drift basins, respectively.

decline in both cases, although for rock basins this amounts to only about 30 per cent, whereas over a much narrower range of lake areas the drift basin ratios fall by nearly 80 per cent. Thus it is clear that depth does not increase in proportion to the horizontal dimensions, especially in deposits of glacial drift. Whether this low *mean depth/area* ratio is an original feature of drift lakes, or due to extensive erosion and sedimentation, is difficult to determine. The 29 kettle holes show little apparent relation between mean depth and area, the median depth being about 10 ft for the whole range of lake areas.

It should now be of interest to examine how far the Scottish formulae for the relation between mean depth and lake area can be extrapolated to larger lakes elsewhere, and Figure 5 shows something of this. (The points on the graph are taken from data compiled by Sir John Murray in Volume 1 of the Bathymetrical Survey Reports, with some emendation of the data for North American lakes from papers by Rawson.) It will be seen that the formula for lakes in rock basins fits very well the data for similar but larger lakes in the Alps and the Scandinavian mountains (triangles). Among the largest lakes of the world, however, none reaches an equivalent mean depth, although Baikal and Kosso-gol in mountainous regions of Asia (two uppermost crosses) approach the line fairly closely.

Most of the large lakes of North America which drain to the Gulf of St. Lawrence, Hudson Bay, and the Arctic Ocean, and similar bodies of water in Scandinavia and northwestern Russia (open circles), are intermediate between the regression lines for Scottish lakes in rock and drift basins. While their drainage channels are often greatly obstructed by drift, the great lakes of North America lie mostly in rock basins which were probably not blanketed by glacial deposits to nearly the same extent as were the much smaller Scottish lochs in drift or drift-dammed basins from which the lower line in Figure 5 was obtained. There are however some Canadian lakes (solid circles) which do lie below the line for Scottish drift basins, these being Lakes Winnipeg, Winnipegosis, Manitoba, and Lake of the Woods. All are remnants of the great early post-glacial Lake Agassiz, which covered vast areas of very mild relief in central Canada during the retreat of the ice-sheets, and they must be largely filled by the sediments deposited in that huge inland basin.

The other group of lakes in Figure 5 (open squares) represents basins in the Irish plain, and since these lie in flat glaciated country covered by extensive deposits of drift, they approach more closely to the Scottish drift basins than to the rock basins. Their situation on limestone substrata may perhaps be

responsible for these lakes exhibiting rather greater mean depths than expected from the Scottish regression line, as some subsidence may have occurred owing to solution of the underlying rock.

The shallowest among the large bodies of water shown in Figure 5 is Lake Balaton in Hungary (lowest cross). This unusual lake fills, to a maximum depth of only 36 ft, a shallow depression which is believed to have resulted from settling of surface rocks into a cavity emptied by lava ejection, the long axis of the lake running parallel to a line of local volcanic action.

Maximum depth and mean depth

The logarithmic relation between maximum and mean depth is exceptionally close, the correlation coefficients being 0.95 and 0.92 for rock and drift basins respectively. However, the relation is somewhat different for the two groups of lakes, as may be seen from Figure 6, and the slopes of the two regression lines, though similar, differ significantly at the 1 per cent level. In addition Figure 6 gives the ratio of maximum to mean depth plotted versus the latter. For a lake of conical section this ratio would be 3, but only a few lakes attain it, chiefly among the smaller rock basins. There is among the rock basins a distinct drop in this ratio with increasing size, from about 3.0 in the shallowest to about 2.4 in the deepest, which implies a change from V- to U-shaped cross-sections. Since the glacial erosion of mountain valleys leads to over-steepening of the sides (*i.e.*, the development of a U-shaped from a V-shaped cross-section) and over-deepening of the bottoms (*i.e.*, the formation of lake basins), this decrease in *maximum/mean depth* ratio with increasing size of the rock basins may, as suggested previously, reflect an evolutionary sequence, in which the larger and more U-shaped basins represent the maximum and the smaller V-shaped basins the minimum of glacial erosion.

Among the lakes in or dammed by glacial drift the trend is in the opposite direction, with the smallest basins exhibiting *maximum/mean depth* ratios of about 2.2 and the largest basins ratios of about 2.8. This

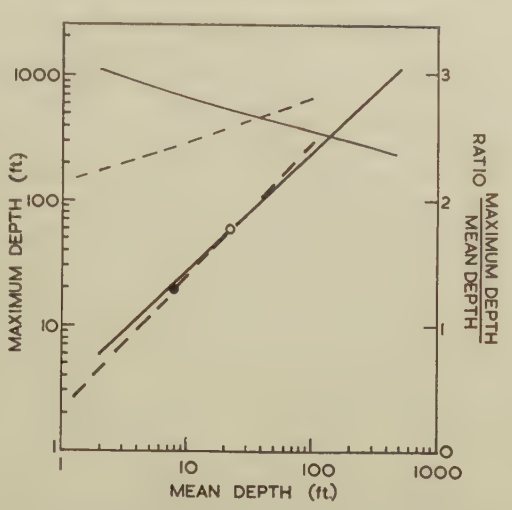

FIG. 6. The relation between maximum and mean depth. The heavy solid line represents rock basins, heavy dashed line drift basins, and circles log means. Ratios are given by lighter lines.

suggests that the smaller lakes have been filled in by erosion and sedimentation to a greater extent than have the larger ones.

On the (logarithmic) average the *maximum/mean depth* ratio is about 2.4 for the drift basins as against about 2.7 for the rock basins. Such a result suggests that erosion and sedimentation of the glacial drift is more effective in producing a U-shaped cross-section than is ice-erosion of the solid rock.

As in the last section, it may be of some value to see how far it is possible to extrapolate the Scottish relationships to larger lakes in other parts of the world, and Figure 7 shows that in fact the largest alpine lakes (triangles) follow quite closely the line for Scottish rock basins. The even larger Laurentian lakes of Canada (open circles) again fall between the lines for Scottish rock and drift basins, and tend on the average to exhibit fairly conical cross-sections. (The circles marked by arrows represent Great Slave Lake, in which the main body of water gives a maximum depth of 535 ft, while a narrow eastern arm—Christie Bay—reaches 2,015 ft.) Those lakes that are the remains of the former Lake Agassiz (solid circles) have rather low *maximum/mean depth* ratios, as might be expected if they are merely the sediment-filled deeps of that huge lake of early post-glacial times. In contrast,

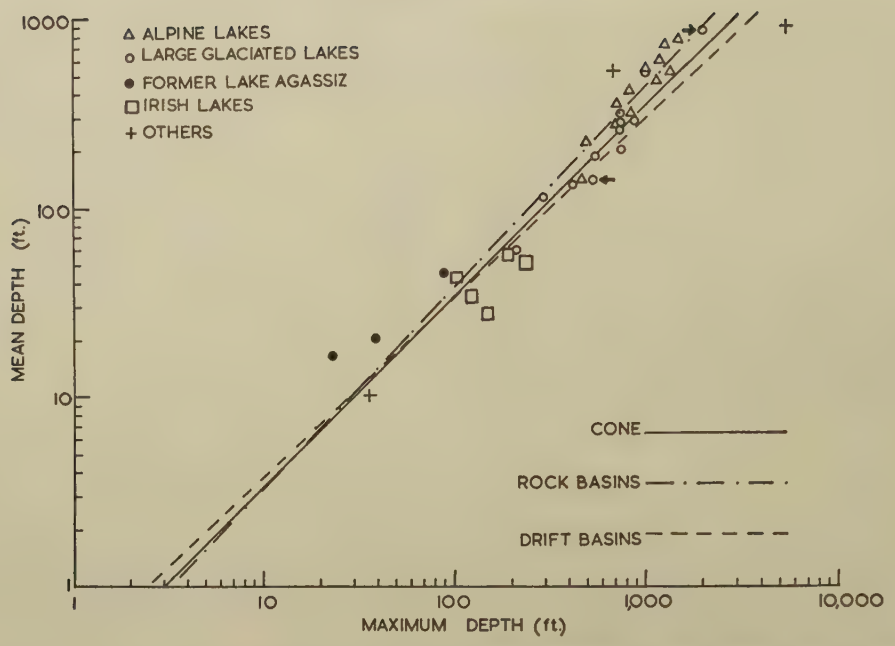

FIG. 7. The relation between mean and maximum depth in some of the largest lakes of the world The solid line represents conical sections, the dashed line is extrapolated from that for Scottish drift basins, and the line of dashes and dots is extrapolated from that for Scottish rock basins.

the Irish lakes (squares) yield somewhat high ratios, with the exception of Lough Neagh. This might perhaps be ascribable to local subsidence, owing to solution of the underlying limestone of the great central Irish plain.

THE RELATION BETWEEN WATER REPLACEMENT AND LAKE DIMENSIONS

The rate of water flow through a lake, which is dependent upon rainfall, evaporation, drainage area, and lake volume, must be of immense limnological importance; for not only are physical properties such as temperature, stratification, and water movements greatly affected, but also chemical properties, as for instance the concentrations of dissolved ions, gases, and other materials, some of which are of undoubted biological significance. In addition, both sediment and plankton dynamics will be greatly influenced by the rate of water replenishment, so it should obviously be worthwhile to calculate the effect of lake size upon this process.

Such a calculation is illustrated on a semi-

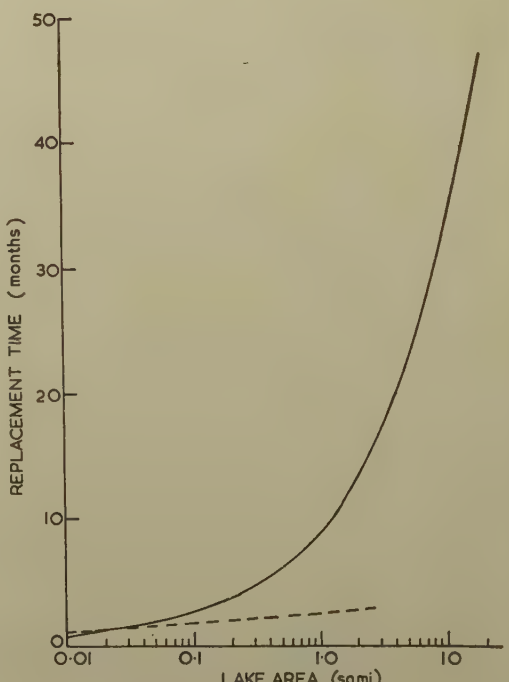

FIG. 8. The time required for complete water replacement within a lake, as a function of lake area. The solid line represents rock basins, the dashed line drift basins.

logarithmic basis in Figure 8, which shows the time for complete water replacement in Scottish lakes of various sizes, assuming 50 inches of rainfall and 15 inches evaporation —the average figures for Scotland as a whole (Glasspoole 1933, Penman 1950). (Incidentally, there is little apparent correlation of basin size with altitude among the lakes surveyed, so that no great differences in average rainfall are to be expected on this account.)

It is clear from Figure 8 that on the average (and neglecting stratification) the smaller lakes exhibit a complete renewal of their waters every few weeks, while for the largest Scottish rock basins the replenish-

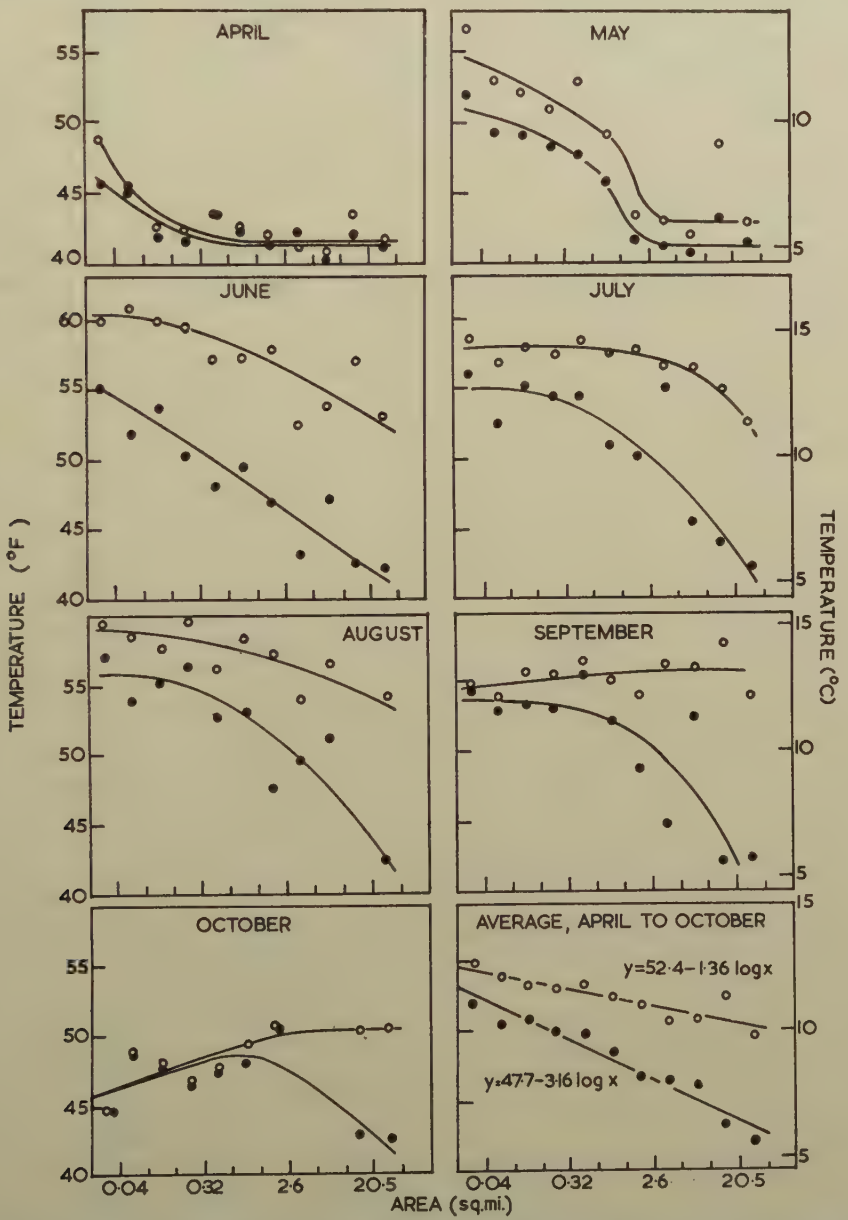

FIG. 9. Monthly temperature of surface and bottom waters in Scottish lakes in relation to lake area. Open circles represent surface temperature, solid circles bottom temperature. The lines have been fitted by eye. The area scale is logarithmic.

ment period may cover some years. Because they are much shallower than rock basins of similar size, the largest drift basins as a rule replace their waters very much more quickly; but in the case of the very smallest basins this position tends to be reversed, owing to the relatively smaller drainage areas of the drift basins in this size range.

WATER TEMPERATURE AND LAKE DIMENSIONS

The temperature of a body of water is of course of great hydrobiological importance, and also affords a useful indication of the degree of stratification. Figures 9 and 10 show water temperatures at the surface and near the bottom in relation to the area and mean depth of the basin (on logarithmic

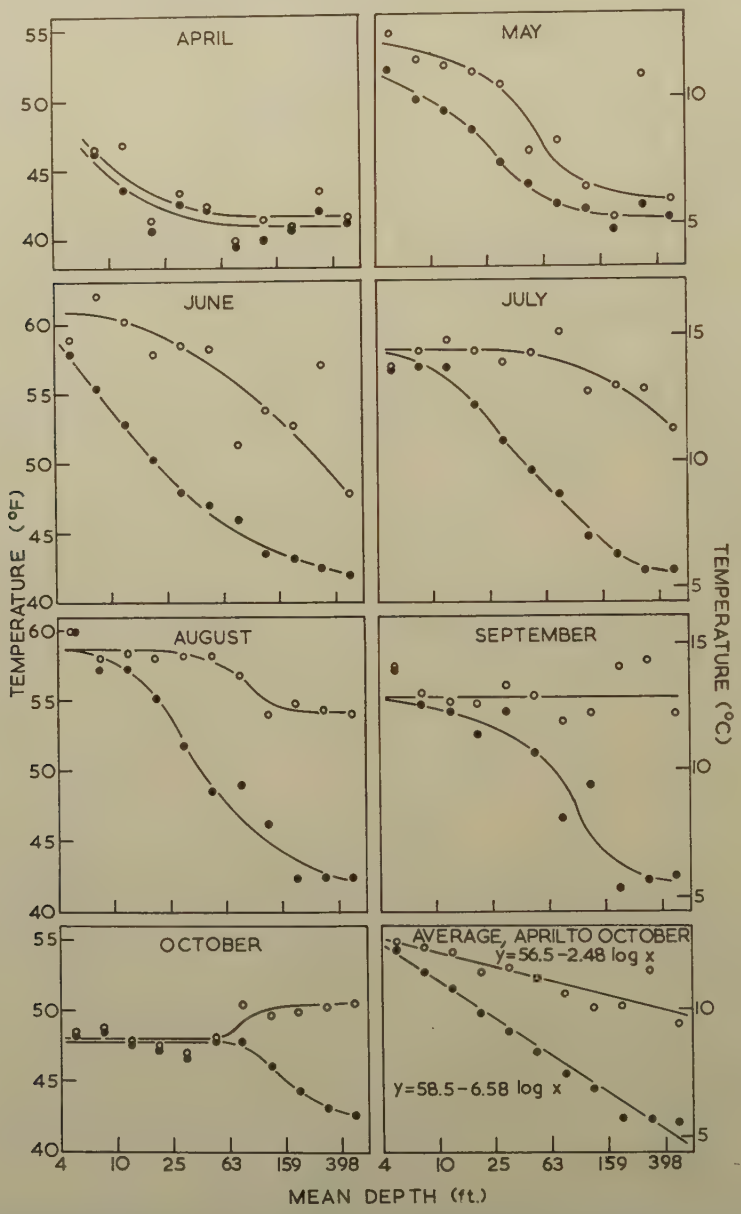

FIG. 10. Monthly temperature of surface and bottom waters in Scottish lakes in relation to mean depth. Open circles represent surface temperature, solid circles bottom temperature. The lines have been fitted by eye. The depth scale is logarithmic.

scales). They are based on data from some 370 lakes—mainly rock basins—for which the formula relating the two dimensions is $\log y = 0.397 \log x + 1.545$ (cf. Table 3). Since the monthly curves have been fitted roughly by eye, and the groups of lakes were different from month to month over the several years of the survey (the temperature profiles of most lakes being examined only once), the results can only be taken as rather approximate. Nevertheless they appear to give a reasonably clear picture of seasonal temperature changes in relation to lake dimensions, which in view of the importance attached to the temperature cycle may be of some general interest.

It is evident from Figures 9 and 10 that by April the lakes are beginning to warm up and to stratify, with the smallest and shallowest lochans reaching temperatures of 45–50°F (7–10°C), while the largest and deepest ones remain at about 41–42°F (ca. 5°C). By May stratification is well advanced in all the lakes, with the smallest lochans reaching about 55°F (13°C) at the surface while the largest lochs are still cold at about 43°F (6°C). From June to August the small lochans tend to maintain their surface temperatures a little above 55°F (13°C), while the big lochs warm up gradually to this temperature. Figure 10 shows that bottom temperatures in the shallowest lochans (mean depth about 5 ft) are at this time only a fraction of a degree lower than those at the surface, while in the deepest lochs (mean depth greater than 250 ft) they remain around 42°F (4–5°C). By September the small lochans have begun to cool down, and by October surface temperatures are falling in all the lakes, while lakes up to about 0.5 mi² area and 50 ft mean depth are nearly isothermal. At this time the large lakes are slightly warmer than the small ones, and remain distinctly stratified; in the case of the largest lake, Loch Ness, stratification does not break down completely until toward the end of November.

Over the period from April to October (roughly coincident with the growing season) average surface and bottom temperatures vary in an approximately linear way with the logarithms of lake area and mean depth, as shown by the graphs and formulae in the bottom right-hand corners of Figures 9 and 10. Average surface and bottom temperatures in the smallest lochans are about 55°F (13°C) and 53–54°F (12°C), respectively, while in the biggest lochs the range is from 50°F (10°C) at the top to about 42°F (5–6°C) at the bottom. Expression of these semi-logarithmic relationships on arithmetic scales shows that the temperature decrease per unit increase of lake size and depth is much greater among the smaller lakes; in the case of mean depth the two arms of the curves may be said to join in the vicinity of 40 ft.

IGNITION LOSS OF PROFUNDAL MUDS

Since the organic content of profundal muds has sometimes been taken as an indication of lake productivity, it seemed worthwhile to see whether the ignition loss of these muds (roughly proportonal to organic content in noncalcareous deposits such as those of all but a very few Scottish lochs) could be related to lake dimensions. Accordingly a series of muds sampled by the Lake Survey from the deepest parts of 54 lakes was obtained (through the courtesy of the Keeper of Natural History at the Royal Scottish Museum in Edinburgh), and then dried at just above boiling point and ignited at incipient to dark red heat. Ignition loss ranged from 4 to 70 per cent dry weight, with the median at about 20 per cent and probably corresponding to an organic carbon content of between 5 and 10 per cent. No difference was discernible between the organic content of muds from large and small or from deep and shallow lakes. For instance the highest value came from Littlester Loch, with a mean depth of 5 ft, while the fourth highest (41 per cent) came from Loch Morar, with a mean depth of 284 ft. In contrast the lowest value came from Loch Suainaval, with a mean depth of 109 ft, and the third lowest (6 per cent) from Boardhouse Loch, with a mean depth of 6 ft.

CONCLUDING REMARKS

From this brief summary of the Scottish Bathymetrical Survey's work in physical limnology it will be evident that Sir John Murray, Mr. Laurence Pullar, and their associates have provided a most detailed

and extremely valuable background for further limnological and especially hydrobiological studies in Scotland. While some work was done at the same time on the plankton and macro-vegetation of the lakes, rather little was published in the Lake Survey reports. The plankton collections are maintained in the Royal Scottish Museum, along with the samples of bottom deposits and many of the original field notebooks of the survey.

REFERENCES

GLASSPOOLE, J. 1933. The rainfall over the British Isles of each of the eleven decades during the period 1820 to 1929. Quart. J. R. Met. Soc., **59**: 253–260.

HAYES, F. R. 1957. On the variation in bottom fauna and fish yield in relation to trophic level and lake dimensions. J. Fish. Res. Bd. Can., **14**: 1–32.

MORTIMER, C. H. 1952. Water movements in lakes during summer stratification; evidence from the distribution of temperature in Windermere. Phil. Trans. B, **236**: 355–404.

———. 1953. The resonant response of stratified lakes to wind. Schweiz. Zeitschr. Hydrol., **15**: 94–151.

MUNK, W. H. 1941. Internal waves in the Gulf of California. J. Mar. Res., **4**: 81–91.

MURRAY, J., AND L. PULLAR. 1910. Bathymetrical survey of the Scottish freshwater lochs. 6 vols., Edinburgh.

PENMAN, H. L. 1950. Evaporation over the British Isles. Quart. J. R. Met. Soc., **76**: 372–383.

RAWSON, D. S. 1953. The standing crop of net plankton in lakes. J. Fish. Res. Bd. Can., **10**: 224–237.

———. 1955. Morphometry as a dominant factor in the productivity of large lakes. Verh. Int. Ver. Limnol., **12**: 164–175.

REVELLE, R. R. 1939. Sediments of the Gulf of California. Bull. Geol. Soc. Amer., **50**: 1929.

TALLING, J. F. 1957. The phytoplankton population as a compound system. New Phytol., **56**: 133–149.

Seasonal Characteristics of Two Saline Lakes in Washington

G. C. Anderson

Department of Zoology, University of Washington, Seattle

ABSTRACT

The limnology of two highly saline lakes in Washington was studied in relation to the physical and chemical conditions which influence the growth and distribution of phytoplankton. The lakes were sampled at frequent intervals for a period of more than a year in 1950 and 1951. Morphometric conditions were determined, and routine sampling included measurements of temperature, transparency, oxygen, pH, alkalinity, phosphate, chlorophyll, and quantitative samples of phytoplankton. Lake Lenore, the less saline of the two (T.D.S. 14 g/liter), is shallow and was unstratified during the investigation. Dissolved nutrients were high and showed erratic variations during the summer. The phytoplankton population was taxonomically simple and was made up mainly of two species of diatoms—*Amphora* sp. which formed the spring bloom, and *Chaetoceros elmorei* which made up the late summer bloom. Soap Lake is relatively deep, meromictic, and saline (T.D.S. 35 g/liter). Temperature conditions were dichothermic, and the nutrient content was high, especially in the monimolimnion. A winter maximum and a summer minimum was observed in the phytoplankton population, and a change in the biota has been noted during dilution of the lake in recent years. Indication of grazing by zooplankton was found in both lakes.

INTRODUCTION

Seasonal information on saline, desert lakes is rather meager, and apart from the work contributed by Young (1924) in Devils Lake, by Hutchinson (1937) on lakes in Nevada, and by Rawson and Moore (1944) on Saskatchewan lakes, little is known of these lakes in North America. Most limnological work in arid regions has been carried out by expeditions to places that are not readily accessible. Due to time limitations, little has been learned of seasonal events, and hence comparison with other types of lakes intensively studied has been superficial. The present paper is confined to seasonal fluctuations of physico-chemical factors and of the phytoplankton populations.

There are many lakes of varying degrees of salinity in the easily accessible Columbia Basin of Central Washington, and for the present study, two of the more saline lakes were chosen for the study of seasonal events. Soap Lake is the terminal one in a chain of lakes which lie in the old river bed of the Columbia River in the Lower Grand Coulee. Lake Lenore lies to the north. The Grand Coulee is a basalt canyon formed by the flow of the Columbia River during glacial times. Upon disappearance of the ice, the river returned to its former drainage system, the present location of the Columbia River, and the Grand Coulee became dry except for the chain of lakes now present (Bretz 1932).

Both Soap Lake and Lake Lenore are highly alkaline, the waters of which are composed chiefly of the sodium salts of sulfate, bicarbonate, carbonate, and chloride (Table 1). A seepage inlet at the north end of Lake Lenore supplies water from the relatively fresh but hardwater lakes lying in the northern section of the Lower Grand Coulee. Lake Lenore has no outlet, however, and Soap Lake has neither a surface inlet nor outlet. It is believed that an underground connection is present between the two lakes, but the significance of this is not known (Mundorff and Bodhaine 1954). It becomes apparent from this that the salt content of the lakes is due to the absence of outlets. A gain of water occurs by drainage from the surrounding cliffs and plateaus especially during the spring runoff, and loss of water takes place chiefly by evaporation. For this reason the salt content has increased since the origin of the lakes. In recent years, there has been a

TABLE 1. *Mineral analyses of the surface waters of Soap Lake and Lake Lenore, November 1950 (mg/liter)*

Analyses by United States Bureau of Reclamation, Denver, Colorado

	Soap Lake	Lake Lenore
Total solids	35280	14080
Calcium	20.6	10.2
Magnesium	8.2	13.8
Sodium	11868	5129
Potassium	1134	437.9
Carbonate	6870	3000
Bicarbonate	5209	3544
Sulfate	6240	2112
Chloride	5467	1438
Nitrate	8.7	8.7

substantial additional flow of water into Soap Lake, which has been ascribed collectively to increased precipitation, rise in ground water level, and irrigation canal leakage. Pumps have been installed by the U. S. Bureau of Reclamation on both lakes to remove the excess water, and as a result the lakes have suffered a considerable decrease in salinity.

Lake Lenore is an elongate shallow body of water. Summer stratification is weak and transitory due to the shallow depth and mixing by wind. The bottom of the lake is rocky for the most part, and aquatic plants around the shore are sparse. Some saltgrass (*Distichlis stricta*) and Nevada club-rush (*Scirpus nevadensis*) have been reported (Scheffer 1946).

Soap Lake is a meromictic lake. Autumn and spring circulation followed by summer thermal stratification occurs in the upper part of the lake (mixolimnion), but the bottom layer (monimolimnion) never becomes mixed with the upper waters. The bottom layer is composed of a dense yellowish-brown water which contained more than four times the concentration of salts of the upper waters in 1950. The odor of hydrogen sulfide is strong in the bottom water and in the black organic deposits which make up the bottom of the lake. There was no evidence of animal life in these deposits. The aquatic plant population of the littoral zone is scant and consists entirely of emergent forms (Scheffer 1946). Saltgrass (*Distichlis stricta*), Nevada club-rush (*Scirpus nevadensis*), and common toad rush (*Juncus bufonius*) were present.

The writer is indebted to Dr. W. T. Edmondson for constant encouragement and direction during the period of the study and for valuable criticism and advice during the preparation of the manuscript. Thanks are due to Dr. G. W. Comita for collaboration in the field work and for permission to use data from his analyses of O_2, pH, and alkalinity. Dr. R. Thompson of the University of Kansas, Dr. R. Patrick of the Academy of Natural Sciences of Philadelphia, Dr. F. Drouet of the Chicago Museum of Natural History, and Mr. H. E. Sovereign aided in the identification of the phytoplankters. Personnel of the U. S. Bureau of Reclamation at Ephrata, Washington, made available all of their information on these lakes in the form of topographic maps, aerial photographs, chemical analyses, and lake level fluctuations. Financial support and equipment supplied by the Department of Zoology and from Initiative-171 funds of the State of Washington are gratefully acknowledged.

MATERIALS AND METHODS

In each lake a single station was established for all routine sampling. In Soap Lake, the station was in the region of maximum depth. Two locations deeper than the station selected were present in Lake Lenore, but both appeared to be atypical of the lake as a whole.

Morphometric data for Lake Lenore were obtained by sounding with a brass chain. Depth contours were added to an outline map of Lake Lenore taken from the U. S. Bureau of Reclamation, Columbia Basin topographic chart 222-P-5529. Morphometric data for Soap Lake were obtained from the U. S. Bureau of Reclamation, Soap Lake topographic map 222-116-25067.

Sampling of the lakes was carried out at different time intervals depending upon the season of year. During the winter, sampling was done every three to four weeks, while during the period of stratification samples were taken every two weeks as far as was possible.

Water samples were usually collected at

the surface, middle, and bottom of the lakes. This procedure was occasionally modified to suit the existing conditions. At each depth four liters of water were collected in either a two-liter or a four-liter Kemmerer water bottle, drained into glass gallon jugs, and brought back to the laboratory in this condition. From the water in these jugs the various chemical determinations were made and phytoplankton samples taken. Before the water was drained from the sampling bottle into the jugs a sample of water was taken in a 250 cc reagent bottle for the determination of dissolved oxygen. An attempt was made to do the water analyses as soon as possible after the return to the laboratory from the lake. The time before analyses was sometimes quite long due to the distance of the lakes from the laboratory. In some instances the samples were analysed within eight to ten hours after taking and in other instances the samples were left overnight, in which case they were stored in a coldroom at a temperature of 10°C.

The surface temperature of the lake and the air temperature were measured with a laboratory thermometer graduated in degrees Centigrade. A temperature profile of the entire depth of the lake was obtained with a bathythermograph.

The transparency of the lakes was measured with a fully white, 20 cm diameter Secchi disc.

A plankton sample for qualitative purposes was taken with a fine-mesh, usually number 25 bolting silk, plankton net. A vertical tow from about five meters depth was the usual procedure. The sample was preserved in approximately 4 per cent formalin.

Dissolved oxygen was determined by the unmodified Winkler method (American Public Health Association 1946). The manganous sulfate, alkaline iodide, and sulfuric acid reagents were added immediately after the sample was taken, and the titration was carried out at the laboratory. Large amounts of acid were needed to neutralize the samples, and because a standard amount was not added each time, corrections were not made for the volume of reagents introduced. With the addition of acid, a violent bubbling took place due to the high carbonate content. Because of these difficulties the oxygen values are open to question.

The hydrogen ion concentration was measured with a Beckman pH meter. Alkalinity was determined by titrating 10-ml samples of lake water with 0.5N sulfuric acid to the phenolphthalein and methyl orange endpoints. Differentiations of the alkalinities due to hydroxide, normal carbonate, and bicarbonate was done according to the American Public Health Association (1946, Table 4, page 9). The results are expressed as mg/liter $CO_3^{=}$ and HCO_3^{-}. Small quantities of hydroxide were recorded infrequently, the significance of which is not known, but may have been due to technical error.

Phosphate concentration was determined by the method described by Robinson and Thompson (1948). Standards were not run with every series of determinations, but repeated calibrations showed that under the conditions used, the variation was unimportant. The method was modified somewhat because of the alkalinity of the lakes. One ml of concentrated sulfuric acid was added before the addition of reagents to lower the pH to 4.2, the point of maximum color development. It was subsequently found that dilution of the samples was necessary to give true values, and corrections were applied to all the data in this respect. The presence of possible interfering substances in the monimolimnion of Soap Lake make those values questionable.

Nitrate determinations were attempted with the strychnidine method (Zwicker and Robinson 1944), but it was found that variability was great and standard amounts added to lake water could not be recovered with any degree of precision. Evidently the strychnidine reagent does not work in highly alkaline waters, and hence special methods will have to be developed for these lakes.

Chlorophyll determinations were made according to the method outlined by Edmondson and Edmondson (1947). One liter of water, or less, depending upon the quantity of plankton present, was filtered through

Schleicher and Schuell number 576 analytical filter paper. The entire filter paper was ground with purified sand in a mortar with 80 per cent acetone. The mortar was allowed to stand at least 15 minutes in the dark, after which time it was filtered through a sintered glass filter into Klett tubes. The filtrate was made up to 10 ml and then read in the Klett colorimeter using filter K66. Edmondson and Edmondson (1947) have shown that the K66 filter adequately measures chlorophyll *a* with a possibility of interference from chlorophyll *b* and chlorofucin.

Phytoplankton samples were prepared by centrifuging 50-ml samples of water in a clinical centrifuge at a speed of approximately 1500 rev/min for 10 minutes. The water was drawn off carefully so that only 1 or 2 ml remained. The remaining solution was thoroughly mixed and transferred to a vial where it was fixed in Transeau's solution (6 parts water, 3 parts 95 per cent alcohol, 1 part formalin). It was found that when samples were mounted on a slide with cover glass, a violent swirling took place which made it impossible to count accurately. This was overcome by increasing the viscosity of the solution with the addition of a few drops of glycerine to each sample. The samples were counted using a method similar to that described by Lackey (1938).

The volume of each species was calculated. The necessary measurements were made with the use of an ocular micrometer, and, by the application of formulae for the volumes of bodies which the species most nearly represented in shape, the volumes were estimated. In this manner the total volume of phytoplankton was calculated.

LAKE LENORE

Morphometry

Lake Lenore is elongate, with a length of 9.2 km and a maximum width of 0.9 km. The maximum depth is 11 m and the mean depth is 6.5 m. Details of the morphometry are given in Table 2.

TABLE 2. *Morphometric features of Lake Lenore*

Depth (m)	Area (ha)	Volume (m^3)
0	556	
		5,408,000
1	526	
		10,128,000
3	487	
		9,196,000
5	433	
		7,207,000
7	292	
		3,468,000
9	78	
		640,000
11	3	
Total		36,042,000
Mean depth 6.5 m		
Maximum depth 11.0 m		

Physical and chemical conditions

Temperature. Thermal stratification in Lake Lenore was at most times absent (Fig. 1). With the disappearance of the ice cover in late February the lake was homothermal at approximately 0°C. During the period of spring heating, a temperature gradient was established and was most pronounced in the middle of May with a surface temperature of 14.2°C and a bottom temperature of 5.6°C. After this, the temperature gradient was gradually reduced, and by the end of July the entire lake was homothermal at 22°C. The lake remained homothermal until the appearance of an ice cover in January, 1951.

The annual heat budget was 16,110 g-cal/cm^2 of lake surface. The period of heating used for the calculation was the minimum weighted mean winter temperature of −0.49°C on February 18, 1950, to the maximum weighted mean summer temperature of 22.2°C on August 27, 1950. The heat budget for Lake Lenore is of limited value and is not comparable with those of large, deep lakes. Because the top and bottom waters attain the same maximum temperature, there must be much heat lost to the bottom mud which would therefore render the value of the heat budget minimal in comparison with other lakes.

Transparency. The range of transparency was large (Fig. 1), from a minimum of 1 m on March 26, 1950, a period when the water was turbid due to spring inflow from the melting snow of the surrounding cliffs, to a maximum of 8.5 m in midsummer and

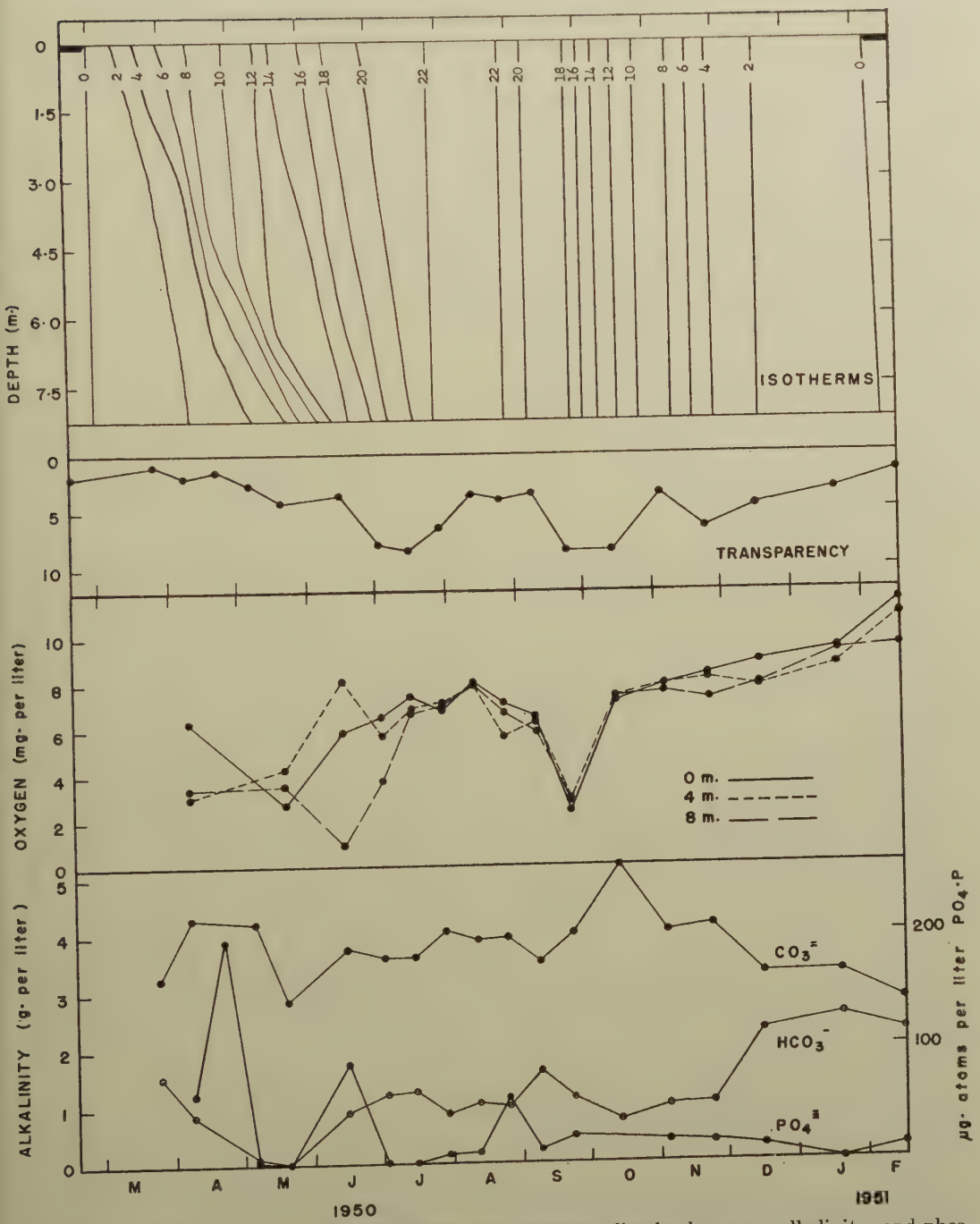

FIG. 1. Seasonal variations of temperature, transparency, dissolved oxygen, alkalinity, and phosphate in Lake Lenore, February 1950 to February 1951. Alkalinity and phosphate values are weighted averages for the entire lake.

autumn. The arithmetic average for the year was 4.4 m.

Dissolved oxygen. As would be expected because of the limited thermal stratification, there was likewise a limited stratification of dissolved oxygen (Fig. 1). The period of maximum oxygen stratification was in June which coincided with the maximum thermal

gradient. The oxygen concentration in the bottom waters reached a minimum of 0.9 mg/liter on June 15 but increased shortly after this due to the mixing of the entire lake. There was a sudden drop in oxygen concentration at all levels on September 23 which, as will be discussed later, coincided with a sudden almost complete disappearance of the phytoplankton population. This was followed by a rise in concentration and maintenance of a constant oxygen concentration throughout the winter. There was another slight increase in February, 1951.

It is difficult to form an idea of the trophic nature of Lake Lenore from the oxygen conditions. Calculation of the hypolimnetic oxygen deficit is not possible because stratification was not established.

Hydrogen ion concentration. There was little vertical or seasonal variation of the pH in Lake Lenore. A minimum of pH 9.69 was observed in the 4 to 8 m depth zone on June 15, 1950, and a maximum of pH 10.02 was obtained at the surface on April 22, 1950. This small range of pH demonstrates the highly buffered nature of the water.

Alkalinity. The alkalinity of Lake Lenore is made up of two fractions, bicarbonate and carbonate. There was little vertical stratification of the alkalinity so that the weighted averages of all the depth zones of the bicarbonate and carbonate illustrate adequately the seasonal fluctuations (Fig. 1).

The bicarbonate was at most times in lower concentration than the carbonate. There was a reduction to zero of the bicarbonate in the spring, which was followed by a rise and then a fairly steady state of approximately 1000 mg HCO_3^-/liter throughout the summer and fall. During the winter there was a further increase to 2500 mg HCO_3^-/liter.

The carbonate remained high at all times. A drop to 2800 mg $CO_3^=$/liter in May, and an increase to 5200 mg/liter in October followed by a gradual decrease during the winter were the salient features of the seasonal carbonate cycle. There were times when a decrease in the bicarbonate concentration was accompanied by an increase in the carbonate concentration. This is probably a result of the conversion of bicarbonate to carbonate due to the removal of CO_2 by photosynthesis.

Phosphate. There was little vertical stratification of the phosphate so that only the weighted average of all depths is illustrated (Fig. 1). The phosphate concentration was very high in early spring (195 μg-atoms/liter) but decreased rapidly almost to zero in May. This was followed by a rise in June and a further decrease to almost zero in July and August. Toward the end of August an increase of short duration occurred. During the remainder of the study, the phosphate concentration fluctuated slightly around 20 μg-atoms/liter. On only one date, June 15, was there considerable vertical stratification, with a minimum of 2.8 μg-atoms/liter at the top and 141.6 at the bottom. Thus, chemical stratification coincided with the period of the maximum thermal gradient. It was not possible to calculate the rate of phosphate accumulation in the bottom waters because of complete mixing after June 15.

The great fluctuations in phosphate concentration indicate either a high phytoplankton production or a storage of phosphate by phytoplankters in excess of that required for growth or possibly a combination of the two. *Nitzschia* and *Chlorella* (Ketchum 1939, Scott 1945) can store phosphorus in excess of immediate requirements, and the same phenomenon has been shown for *Asterionella* (Lund 1950). Einsele (1941) described the storage of large quantities of phosphate by the Schleinsee phytoplankton with no increase in the population.

Phytoplankton

The phytoplankton of Lake Lenore is taxonomically simple in regard to numbers of species present (Table 3): 15 genera, 18 species, and 2 miscellaneous categories occurred. One miscellaneous group is composed of small blue-green and green algae, the other is composed of diatoms.

The three indices of the standing crop—chlorophyll concentration, cell number, and cell volume—showed the same general seasonal trends, although the magnitude of

TABLE 3. *Volume calculations of the seasonal and vertical distribution of the species of phytoplankters in Lake Lenore, March 1950 to February 1951*

For each species and date the upper figure represents volume at 0 m, middle-4 m, and lower-8 m. Multiply the values shown by 10^3 to get μ^3/ml.

	1950															1951	
	Mar. 4	Apr. 8	Apr. 22	May 20	June 15	July 15	July 29	Aug. 12	Aug. 25	Sept. 8	Sept. 23	Oct. 14	Nov. 4	Nov. 24	Dec. 16	Jan. 20	Feb. 17
Chrysophyta																	
Asterionella formosa									10.8	6.7							
		8.7		4.0					1.3	7.4	2.0					1.3	
		2.0		35.7					26.9		1.3						
Chaetoceros elmorei (vegetative cells)							43.6	15.0	213.7	694.1	28.6		4.1		8.2	6.8	
							35.4	34.0	183.7	295.3				13.6	8.2		
							15.0	61.2	530.8	458.7	9.5		12.2	13.6	10.9	12.2	
Chaetoceros elmorei (statospores)	1.4							46.8	54.9	42.0	4.1		8.8	31.2	5.4	43.4	
								116.6	15.6	158.7	5.4	8.8		22.4	25.8	45.4	10.8
									71.2	118.6		1.4	27.1	36.6	16.9	87.5	
Cyclotella				2.4					12.1			17.0					
				36.3					4.8	26.7							
									9.7	12.1							
Amphora	0.8	127.2	763.4	1.5	5.1	0.9		0.4			0.6		4.4	12.5	22.5	27.3	12.4
	3.0	35.4	405.0	14.2	1.3	0.4	0.1	0.4				0.4	0.3	7.8	42.8	19.3	40.3
		24.4	419.8	133.1	1.3		0.4	0.2	0.4	20.0		0.7	4.4	9.3	24.0	29.6	
Eunotia															5.1		
Fragilaria crotonensis				2.0													
								2.0									
Melosira distans				1.1		22.6								2.2			
				18.3						35.5							
									79.7								
Navicula		3.9	25.1	0.2	1.2	1.9	1.4	3.4	1.7	7.2	1.0	1.1	1.1	6.9	0.6	8.0	11.2
	0.7			1.4	2.1	0.8	4.6	0.6		5.2	0.5			0.5	1.7	5.9	10.3
		1.0		1.1	0.6		1.9	51.5	47.0	8.0			1.0	1.7		9.9	
Nitzschia						0.6											
					0.4												
Tabellaria flocculosa		6.5													12.0		
Miscellaneous diatoms		1.9												1.3			
				1.3		1.3				8.8							
Chlorophyta																	
Gloeocystis							5.0	0.1					0.1				
							5.2										
							2.1				2.2	0.1					
Cyanophyta																	
Oscillatoria		8.3	15.5														
		17.0														8.3	
Spirulina major																0.1	
								0.1									
								0.2		1.0							
Miscellaneous	2.0	10.1	18.9	0.2	6.8	3.2	0.7	2.0			1.1		1.3	0.4	1.1	9.0	27.7
	1.8	13.9	4.9	1.8	3.4	2.9	0.7	3.6		7.0	0.5		0.5		2.7	8.1	17.6
		13.7	5.6	1.3	1.4		0.2	0.9	1.6	12.8			1.6		0.5	3.2	
Weighted average	7.3	85.6	527.2	81.8	8.0	11.7	41.0	195.9	366.4	618.2	16.7	9.8	18.3	50.8	67.7	101.2	71.7

the fluctuations relative to each other was somewhat different (Fig. 2). There were two major peaks in the standing crop, the first occurring in the latter part of April and the second in August and September. The cell volume reached the same magnitude in both peaks whereas the cell number was much reduced during the second peak.

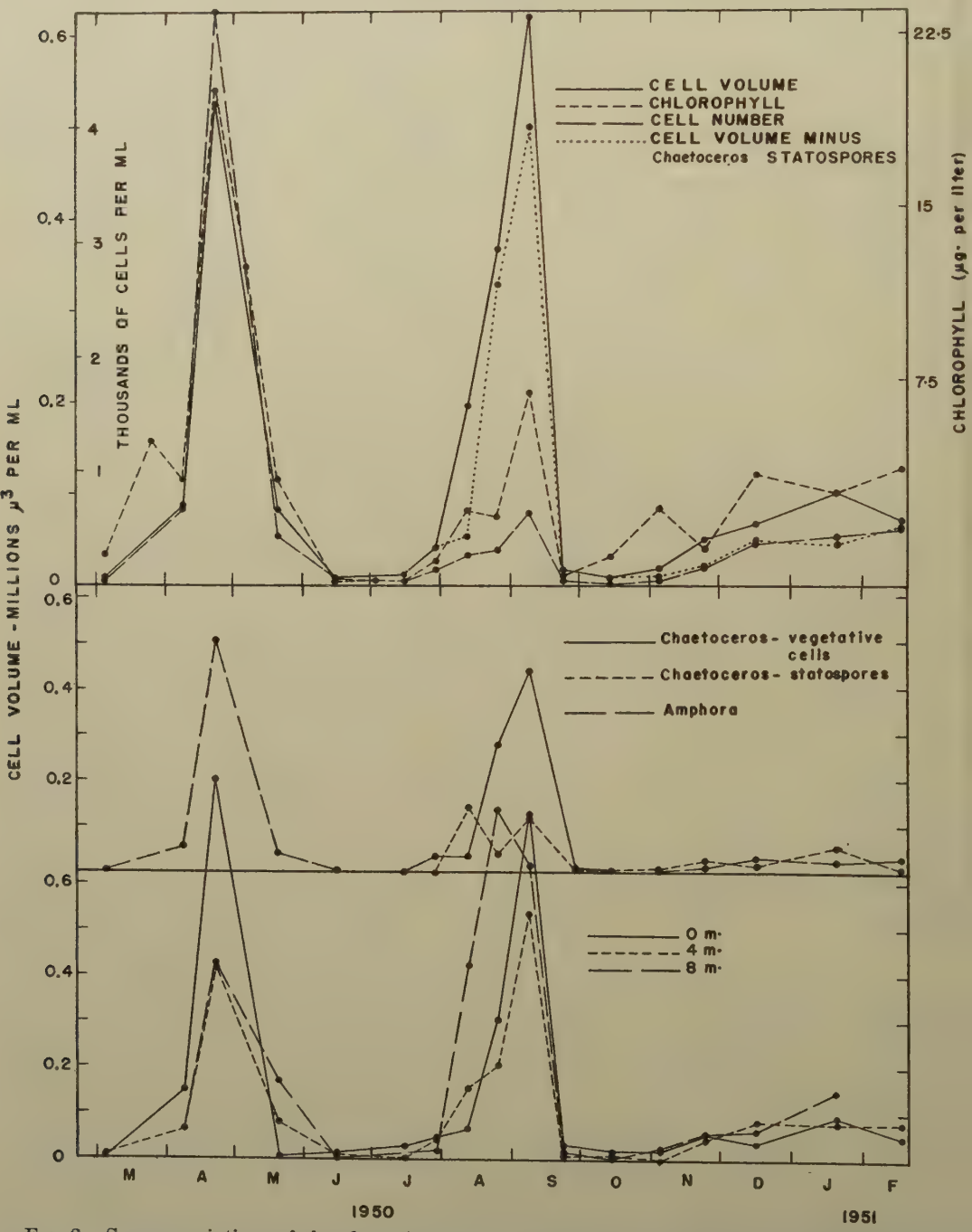

FIG. 2. Season variations of the phytoplankton population in Lake Lenore, March 1950 to February 1951. The upper and middle panels show weighted averages for the entire lake. The lower panel illustrates vertical distribution.

The chlorophyll concentration was not as great during the second peak as during the first, but was in an intermediate position between the cell volume and cell number. The reason for the discrepancy between cell number and cell volume during the fall bloom is that the dominant phytoplankter was a relatively large species, *Chaetoceros elmorei*, whereas the spring bloom was made up of a relatively small form, *Amphora* sp. After the sudden drop of the phytoplankton towards the end of September, the population gradually increased and passed the winter with little fluctuation.

The spring bloom occurred during the period of early spring heating of the lake and reached a peak when the surface temperature was at 9.4°C (Fig. 1). Transparency was reduced during the bloom. Previous to and during the bloom, phosphate and bicarbonate concentrations were high. During the decline of the phytoplankton the phosphate and bicarbonate content dropped almost to zero, the carbonate content decreased somewhat, and transparency increased.

The decline in the standing crop of phytoplankton is not necessarily correlated with the rate of production. In a subsequent discussion it is suggested that the decline in phytoplankton population size was most probably due to grazing by large populations of zooplankters, and that the actual rate of production of the phytoplankton was likely just as great as or greater than during the time of the greatest standing crop. This would explain the reduction in quantity of nutrients present during the decline of phytoplankton density and the increase in transparency of the waters.

The late summer bloom occurred at a temperature between 19° and 22°C. Transparency and phosphate were again reduced. The sudden decline of phytoplankton in September was accompanied by the period of maximum transparency and a large decrease of oxygen in the lake (Fig. 1).

Only the diatoms were of quantitative importance. On each date of sampling they were the dominant group and made up almost exclusively the two major blooms. Rawson and Moore (1944) reported in their study of saline lakes the considerable abundance of diatoms. The green algae appeared during the summer and fall, while the blue-green algae occurred at all seasons.

Amphora sp. and *Chaetoceros elmorei*, the main species of diatoms, together constitute the greatest portion of the phytoplankton population. There were four species of *Navicula* whose occurrence was frequent but contributed little quantitatively. The remainder of the population occurred sporadically and showed no maintained trends of population growth.

The spring bloom of phytoplankton was composed almost exclusively of *Amphora* sp. (Fig. 2). It is possible that this diatom has a fairly limited temperature range. The species was most abundant in spring in the temperature range of 8° to 10°C. With an increase of temperature, the abundance decreased rapidly, and during the summer months *Amphora* was at all times present but in scarcely measurable numbers. It did not begin to increase again until November when the temperature dropped below 10°C. A respectable abundance was maintained throughout the winter. The preceding thermal optimum hypothesis is admittedly speculative and would need controlled laboratory experiments for confirmation.

The diatom comprising the bulk of the late summer bloom was *Chaetoceros elmorei* (Fig. 2). This diatom has been reported only twice in the literature as far as was ascertained. It has been found in Devils Lake, North Dakota (Young 1924), and from 14 saline lakes in Saskatchewan (Rawson and Moore 1944). In Saskatchewan it occurred in lakes which had a range of 400 to 30,000 mg total solids/liter and was particularly abundant in the range 10,000 to 30,000 mg/liter. Lake Lenore and Devils Lake at the time of Young's work had similar concentrations of approximately 13,000 mg total solids/liter. In studies of inland saline waters of Algeria, *Chaetoceros* sp. was found in four localities in which the salinity was relatively low (density 1.004–1.008) (Beadle 1943).

Vegetative cells of *Chaetoceros* began to appear toward the latter part of July at the beginning of the bloom. They increased to a maximum in early September and then suddenly almost completely disappeared by

the end of September. Statospores were formed soon after the appearance of the vegetative cells. Two peaks in the numbers of statospores were observed, the first on August 12, 1950, and the second on September 8, 1950. Statospores remained relatively abundant throughout the winter, whereas vegetative cells were scarce.

In most cases the greatest concentration of statospores was found in the bottom waters. Apparently the statospores have a relatively rapid sinking rate and, if there is little wind mixing, settle rapidly to the bottom. It would appear that they persist in the mud, are occasionally stirred into the water by wind action, and initiate a bloom when conditions once again become favorable for growth.

Although vertical stratification of the phytoplankton existed rarely, there are a few instances of stratification (Table 3). The spring bloom of *Amphora* was concentrated in the surface waters throughout April. In May, during the decline of the bloom, the major part of the population was found in the bottom waters. On June 15, 1950, a time of little thermal stratification, the population was evenly distributed. The next instance of vertical stratification appeared with the *Chaetoceros* bloom. As mentioned previously, the greatest numbers of statospores were usually found in the bottom waters. On one date, August 25, 1950, the vegetative cells were also found in greatest abundance in the bottom water. After this date the phytoplankton population once again became equally distributed and remained so throughout the winter.

TABLE 4. *Morphometric features of Soap Lake*

Elevation (m above sea level)	Area (ha)	Volume (m^3)
329.4	364.49	
		5447686
327.9		
		4929931
326.3	323.95	
		4806120
324.8		
		4434687
323.3	273.91	
		3853150
321.8		
		3106532
320.2	175.47	
		2281125
318.7		
		1684581
317.2	95.48	
		1309396
315.7		
		1088036
314.1	64.66	
		1069277
312.6		
		660326
311.1	38.67	
		517755
309.6		
		423959
308.0	25.69	
		330163
306.5		
		366381
305.0	15.53	
		195096
303.5		
		108804
301.9	4.17	
		26263
300.4		
Total		36,539,268

Maximum depth 27.9 m
Mean depth 10.02 m
Surface level during investigation varied between 327.4 and 328.3 m.

SOAP LAKE

Morphometry

Soap Lake is elongate, with a length of 3.6 km and a maximum width of 1.3 km. The maximum depth is 27 m, the mean depth 7.5 m. Details of the morphometry are given in Table 4.

Physical and chemical conditions

Temperature. The type of thermal stratification found in Soap Lake is temperature inversion or dichothermy. The temperature of the chemocline ranged from a maximum of 3.0° in November, 1949, to a minimum of 0.3°C in May, 1950. The bottom of the monimolimnion had a fairly constant temperature of approximately 5.6°C, and the mixolimnion stratified thermally during summers in much the same manner as holomictic lakes of temperate zones.

The seasonal temperature cycle of Soap Lake is an extremely interesting one (Fig.

3). The study began in November, 1949, when the mixolimnion was undergoing fall circulation. Complete mixing continued until the temperature of the entire mixolimnion had reached a temperature of −1.4°C. The lake froze over in January and remained frozen until the end of February. Little thermal variation occurred in the lake during this period with the exception of a slight increase in depth of the temperature minimum in the chemocline. With the disappearance of ice heating began in the mixolimnion, and stratification became established in the manner of most lakes of moderate depth. A sharp temperature gradient was established at the surface in early April, and the thermocline was gradually depressed to a maximum depth of 8 to 12 m in August.

It is interesting to follow the 0°C isotherm during this period. Winter conditions had left a large portion of the mixolimnion at 0°C and lower, and with the advent of spring stratification much of this water was sealed off between the thermocline of the mixolimnion and the chemocline. Apparently the salinity is great enough that the temperature of maximum density is below 0°C. Seawater of comparable salinity has a freezing point of −2.0°C and the temperature of maximum density is −2.9°C (Thompson 1932). As heating progressed deeper and deeper into the lake the layer of cold water was reduced. The 0°C isotherm disappeared in early July, and at the time of fall mixing the minimum temperature was almost 1°C. Fall mixing began in October and the mixolimnion became hormothermal between 2 and 3°C in December. Meteorological conditions were much less severe in 1950–1951 than the previous winter, and only one-half the surface of the lake had an ice cover. A 0°C isotherm was established but was of short duration, disappearing by the end of February 1951.

A midsummer vertical temperature series with corresponding conductivity measurements on September 9, 1953, was provided by Dr. W. T. Edmondson (Fig. 4). Measurements were made according to the

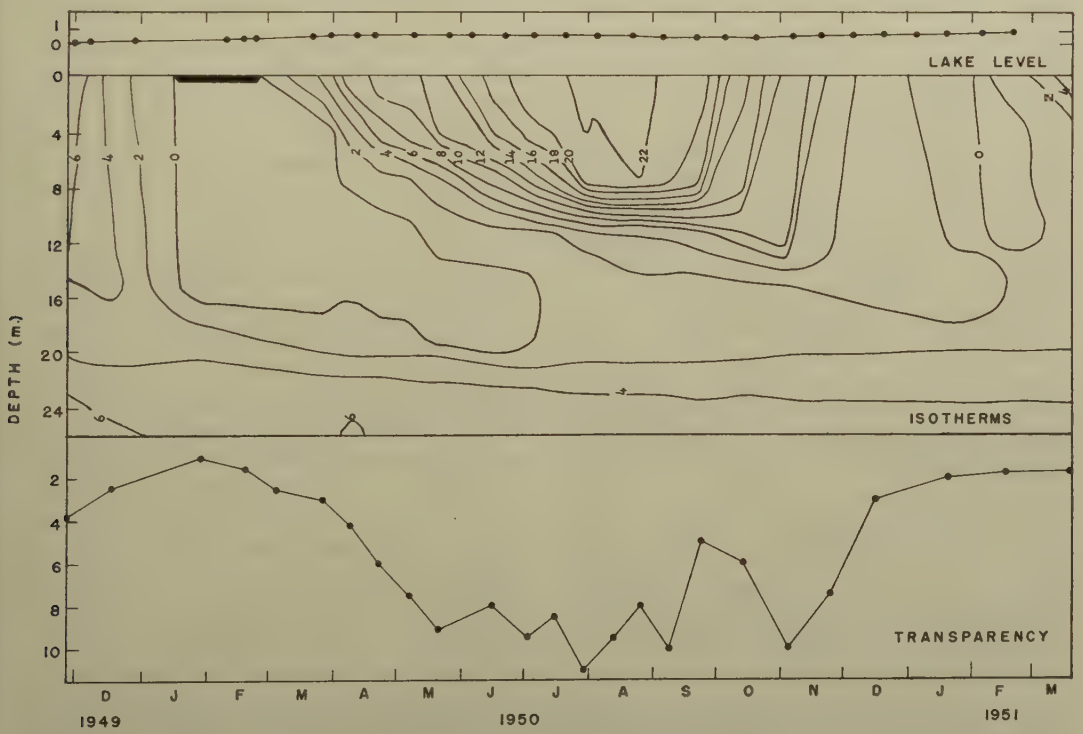

FIG. 3. Seasonal variations of temperature and transparency in Soap Lake, November 1949 to March 1951. Thick horizontal line in isotherm panel indicates duration of complete ice cover.

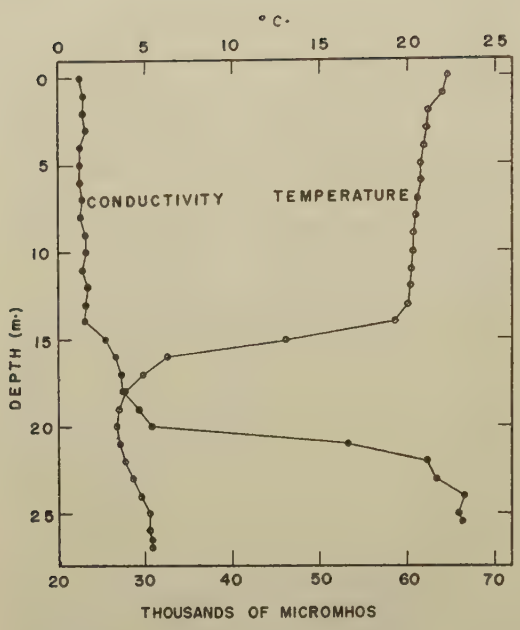

FIG. 4. Vertical variations of temperature and conductivity in Soap Lake, September 9, 1953.

method outlined by Edmondson (1956). Conductivity and temperature were nearly uniform from the surface to a depth of 14 m, the extent of the epilimnion. At 20 m the temperature dropped to 3.4°C, but below this depth gradually increased to 5.4°C at the bottom. The conductivity showed a gradual but small increase from 14 to 20 m, and beyond this depth a more than two-fold increase in conductivity was observed. There was a major difference in the extent of heating in the lake in 1950 and 1953. In September, 1950, the bottom of the epilimnion was at a depth of 8 m and the temperature minimum of the chemocline was 0.6°C, whereas in 1953 a layer of uniform salinity and temperature extended to a depth of 14 m and the temperature minimum of the chemocline was 3.4°C.

The annual heat budget calculated for Soap Lake was 14,902 g-cal/cm² of lake surface. The value is small, especially when one considers the fact that Soap Lake is located in a hot dry desert climate. Also, the lake is completely exposed to winds from a north-south direction and has some degree of, but by no means complete, protection from winds from an east or west direction. This illustrates the remarkable stability of Soap Lake which is of course necessary to maintain meromixis and which has been commented on for other meromictic lakes (Hutchinson 1937).

Transparency. The transparency ranged from a minimum of 1 m on January 28, 1950, to a maximum of 11 m on July 29, 1950 (Fig. 3). There was a sustained winter minimum. Transparency was high during most of the summer and decreased in the latter part of September and October. The arithmetic average for the period studied was 5.7 m.

Total solids. Some records of the total solids concentration in Soap Lake since 1945 are available. U. S. Bureau of Reclamation data show that the approximate average of total solids in the mixolimnion from 1945 through 1950 was 35,000 mg/liter. In 1949 the lake level began to rise, and it was suspected that the lake was being "freshened" by increased precipitation and underground seepage from the surrounding irrigation project. On May 16, 1954, a vertical series of total solids revealed that the average concentration in the mixolimnion was 28,000 mg/liter, which indicated a dilution of 20 per cent. No significant change was detected in the monimolimnion, the maximum recorded being 144,000 mg/liter.

Dissolved oxygen. Little interpretation can be made of the oxygen data owing to the uncertainty of the technique and to the short period of measurements, May 20 to November 4, 1950. Oxygen in the surface waters was generally low, never exceeding 6.6 mg/liter. There was an increase in oxygen concentration in waters of the 10 m depth zone in midsummer which was probably due to photosynthesis below the thermocline. There was no oxygen present at any time below 15 m, and on only two occasions was there even a slight trace of oxygen at 15 m.

Hydrogen-ion concentration. The range of pH was small, which demonstrates the highly buffered nature of the water. A maximum of pH 10.0 occurred in the

mixolimnion on April 22, 1950, and a minimum of 9.4 was recorded in the monimolimnion on August 12, 1950.

Alkalinity. Two fractions, bicarbonate and carbonate, make up the alkalinity in Soap Lake. Little vertical stratification of either fraction occurred in the mixolimnion so that a discussion of the weighted averages will suffice (Fig. 5).

The bicarbonate was at all times lower than the carbonate concentration. The bicarbonate decreased to zero in May, returned to its former level of approximately 2,000 mg HCO_3^-/liter in June and showed little fluctuation during the remainder of the study. The carbonate developed a peak in June and a slight decrease in January. Otherwise, it fluctuated roughly around 8,500 mg $CO_3^=$/liter.

There were large fluctuations in the alkalinity at 20 m depth, but it is believed that this was due to sampling in slightly different levels of the chemocline. The average values at this depth were 4,800 mg HCO_3^-/liter and 24,000 mg $CO_3^=$/liter.

Phosphate. Many of the small irregular fluctuations in the seasonal phosphate cycle of the mixolimnion (Fig. 5) are probably due to technical error (see discussion of methods). The main trend was that of a summer maximum and a winter minimum. This is in agreement with the trend displayed by the transparency (Fig. 3) and chlorophyll (Fig. 6), which indicates a maximum population of phytoplankton and major utilization of the phosphate by the phytoplankton throughout the winter.

The phosphate concentration in the monimolimnion was extremely high, reaching a maximum value of 2,085 μg-atoms/liter. Most of the fluctuations at this depth were probably due to sampling at slightly different levels in the chemocline, because of changes in the lake level.

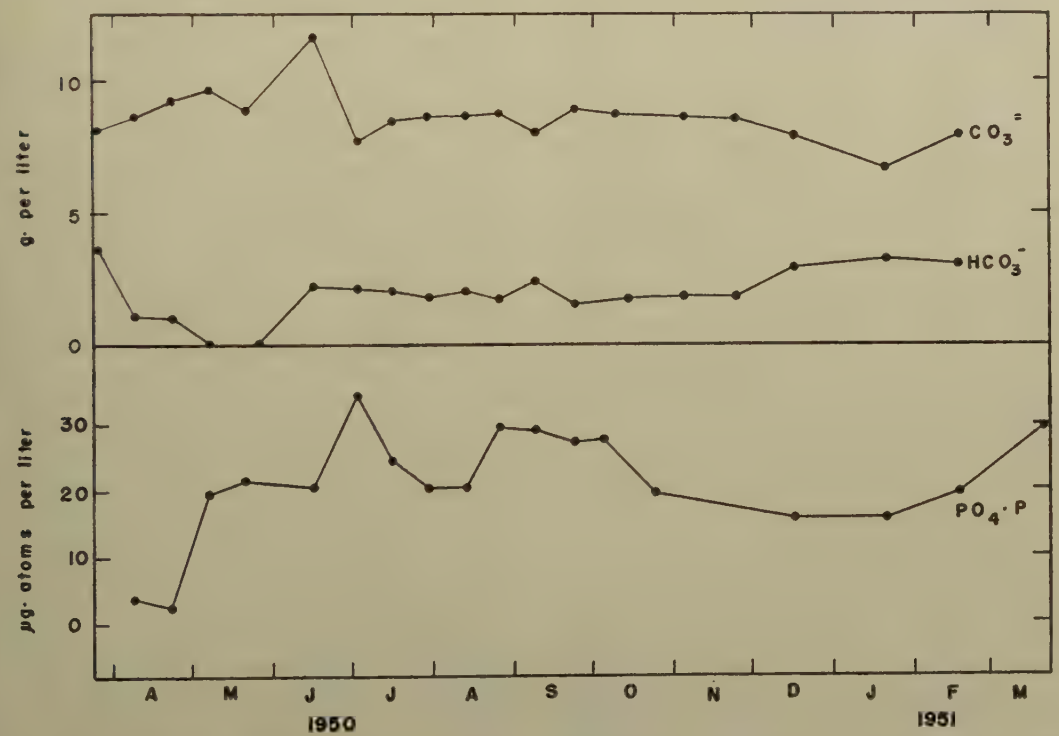

FIG. 5. Seasonal variations of the carbonate, bicarbonate, and phosphate concentrations in the mixolimnion of Soap Lake, April 1950 to March 1951. Weighted averages.

Phytoplankton

Phytoplankton samples were obtained and fixed with Transeau's solution, but it was found after a period of time only unidentifiable fragments remained. A similar degradation of the zooplankton samples by the highly alkaline water occurred. Therefore, the only direct index of the phytoplankton population is the chlorophyll concentration (Fig. 6).

Frequent cursory examinations of fresh samples revealed the paucity of species described for other saline lake (Rawson and Moore 1944). Forms seen and recognized were *Amphora* sp. and *Ceratoneis arcus*. An examination of Soap Lake water obtained in May, 1954, revealed a bloom of *Chaetoceros elmorei*, a species which was not recorded in 1950.

The standing crop of phytoplankton as measured by chlorophyll showed two major peaks during the investigation. In the mixolimnion the surface water demonstrated a peak during both winters, and a minimum from April through November. The population at 10 m also had a peak during both winters, but the first bloom remained in high numbers and did not reach a minimum until July. After this time the seasonal distribution of phytoplankton in the mixolimnion displayed little vertical stratification.

The concentration of chlorophyll in the monimolimnion apparently represents that part of the phytoplankton population which settles to the bottom of the lake in the form of dead or dying cells or in the form of fecal pellets from the zooplankton population. The first peak was reached in the middle of May, thus representing a time lag which might be interpreted as the time required for the settling of cells from the upper water. The minimum did not occur until September and this might indicate a decreased settling rate once the dense water of the monimolimnion was reached. The study was not continued far enough into 1951 to demonstrate a time lag in the build-up of the chlorophyll concentration in

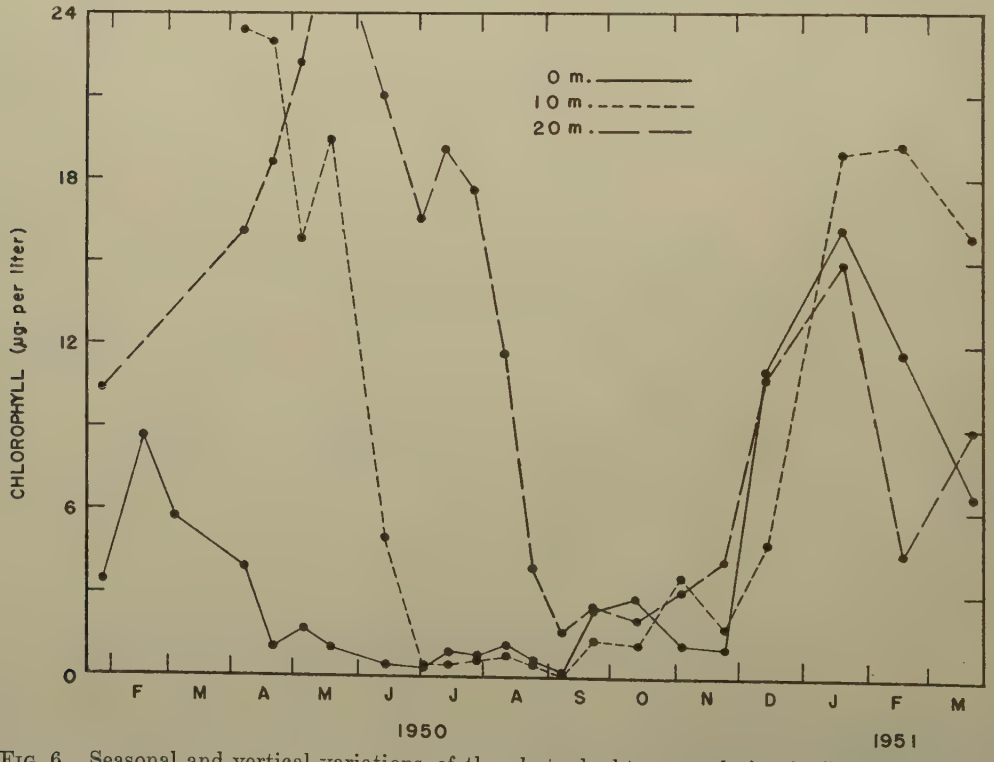

FIG. 6. Seasonal and vertical variations of the phytoplankton population in Soap Lake, January 1950 to March 1951.

the monimolimnion after the 1951 winter peak of phytoplankton in the mixolimnion.

The presence of *Chaetoceros elmorei* in 1954 and its apparent absence in 1950 provides additional information on the salinity tolerance of the species. Rawson and Moore (1944) reported *Chaetoceros* in lakes having total solids concentration of up to 30,000 mg/liter. The next lake in their series had a concentration of 120,000 mg/liter, and *Chaetoceros* was not present. From these data it could be ascertained only that the critical level lay somewhere between these two values. Soap Lake affords the opportunity to come much closer to an estimate of the critical level. In 1950 the total solids averaged 35,000 mg/liter and in 1954, 28,000 mg/liter. *Chaetoceros* was at bloom level of abundance in May, 1954 so that the critical value probably is in excess of 28,000 mg/liter, but of course less than 35,000 mg/liter.

EFFECT OF GRAZING

It has been reported previously that a high production rate of phytoplankton in Soap Lake may have been masked during the spring and summer season due to a large population of grazers (Anderson *et al.* 1955). The zooplankters, *Moina hutchinsoni* Brehm, *Hexarthra* (= *Pedalia*) *fennica* (Levander), and *Brachionus plicatilis* Müller, were in great abundance during the summer period of phytoplankton minimum, and a clear reciprocal relationship was found with the phytoplankton. In Lake Lenore the first spring phytoplankton maximum was followed by a pulse of *Moina* and *Diaptomus sicilis* Forbes, and the late-summer phytoplankton peak was followed by a resurgence of *Moina*, *Diaptomus*, and *Hexarthra*. Thus, it was concluded that the most probable interpretation of the seasonal changes observed in size of the phytoplankton population is its control by the grazing activity of the zooplankton.

In order to demonstrate further the effect of grazing on phytoplankton population density, two attempts were made to measure directly the amount of grazing in July and October, 1955. Two one-gallon jugs of lake water were suspended in both Soap Lake and Lake Lenore. One jug contained the raw lake water and the other contained lake water which had been filtered with number-10 mesh silk net to remove only the zooplankton. In the July experiment zooplankton was added to the unfiltered jug to increase the population approximately twofold, whereas in the October experiment, conditions were not modified in the unfiltered jug. The initial population density was measured by means of chlorophyll concentration, and the jugs were left for a period of a few days. Differences in chlorophyll concentration between filtered and unfiltered jugs indicated the amount of grazing.

In both experiments in Soap Lake, there was evidence that grazing significantly reduced the phytoplankton population size, since a much higher chlorophyll concentration developed in the jug from which zooplankton had been removed. The data are not conclusive for Lake Lenore since both the filtered and unfiltered water contained approximately equal concentrations of chlorophyll at the end of the experiment (Table 5). At present there is no explanation for the apparent failure of the zooplankton to decrease the phytoplankton concentration in the Lake Lenore population. The net increase in chlorophyll of all jugs over the initial concentration can probably be attributed to the added growth of periphytic algae on the glass walls. The data would have more significance had cell counts been made in addition to the chlorophyll measurements but this was not pos-

TABLE 5. *The effect of grazing by zooplankton on phytoplankton population density as shown by gallon jugs containing filtered and unfiltered Soap Lake and Lake Lenore water suspended in each lake for certain intervals of time in 1955*

	Soap Lake		Lake Lenore	
	July 3–July 7 102 hr	Oct. 19–Oct. 25 145 hr	July 2–July 6 99 hr	Oct. 19–Oct. 25 144 hr
Chlorophyll (μg/liter)				
Initial	0.45	3.15	0.30	2.40
Final				
Zooplankton present	0 75	5.10	0.60	3.60
Zooplankton absent	1.80	5.55	0.60	3.45
Zooplankton (no./liter)				
Copepoda			472	105
Cladocera	185	12	90	34
Rotifera	70	154	614	93

sible due to preservation difficulties. Only recently it has been demonstrated that the chlorophyll content of phytoplankton populations may show marked diurnal variations independent of changes in population size due to cell division or death (Yentsch and Ryther 1957).

DISCUSSION

Although Soap Lake and Lake Lenore are similar in origin and are situated in close proximity, the two lakes are quite different in structure and metabolism owing chiefly to the greater depth and salt content of Soap Lake. Soap Lake has permanent stratification, while stratification is virtually absent in Lake Lenore. As a result, Soap Lake has a large store of nutrients in the monimolimnion, a small fraction of which might be mixed with the surface waters during the overturn of the mixolimnion, whereas the transport of nutrients from the bottom waters in Lake Lenore is continuous.

One of the striking similarities found between the two lakes was the lack of variety of phytoplankton organisms, which is evidently a characteristic of highly saline lakes. Although a qualitative list of organisms for Soap Lake was not determined, due to preservation difficulties, frequent examination of fresh samples revealed the paucity of species. A total of only 18 species was found in Lake Lenore. Rawson and Moore (1944) recorded a total of 13 species of algae from Little Manitou Lake, a saline lake in Saskatchewan. Although the lake has a salinity approximately eight times as great as Lenore and is also qualitatively different, it demonstrates the paucity of species occurring in highly saline lakes. In six other lakes in Saskatchewan, of the same magnitude of salinity as Lenore, there was a total of 34 species of algae that were occasional to abundant. Hutchinson (1937) found the same paucity of species in his study of saline lakes in the Lahontan Basin of Nevada. It should be pointed out that the Washington lakes are different from those in Saskatchewan in that the more saline lakes of Saskatchewan are of the sulfate type with usually more magnesium than sodium and are not especially alkaline, having a pH range from 8.0 to 8.9 with the exception of Manitou Lake, which had a pH of 9.5.

The seasonal fluctuations of the total standing crop of phytoplankton were quite different in the two lakes. Lake Lenore followed the pattern found in many described freshwater lakes in that two well-defined major peaks of population density occurred, an early spring and a late summer peak. Soap Lake was unusual in that the one annual peak observed occurred during the winter beneath the ice cover in 1950 and during the coldest part of the year in January, 1951. However, from experimental evidence in Soap Lake and from the sequence of phytoplankton-zooplankton events in both lakes, it appears that the most probable interpretation of the seasonal fluctuations observed is that of control of phytoplankton population size by the grazing activity of the zooplankton.

Since phytoplankton organisms must absorb nutrients for growth, it may be expected that a high concentration of nutrients will ordinarily not be maintained in the presence of a large phytoplankton population, and large increases in the population will usually be accompanied by a decrease in the nutrient concentration. See, for example, the sudden drop in phosphate concentration in Lake Lenore after the occurrence of the two major blooms of phytoplankton (Figs. 1 and 2), and the build-up of phosphate in Soap Lake during phytoplankton minima in midsummer 1950 and early spring 1951 (Figs. 5 and 6). However, the relationship is not always clear. The fact that nutrients are often rapidly released by action of bacteria and other heterotrophic organisms may obscure the relation, and at many times the concentration of nutrients may be a minor factor in governing the size of the phytoplankton population, other factors such as grazing, parasitism, light, temperature, etc., or combinations of the above being the limiting factors. It is possible, at times, that the nutrient supply is in excess and its rate of supply greater than the demand being made upon it by the phytoplankton, and also it has been shown

by several workers that some species of phytoplankton are able to store nutrients greatly in excess of that required and thus continue to grow at times of nutrient deficiency (Ketchum 1939, Scott 1945, Lund 1950, Mackereth 1953).

SUMMARY

Lake Lenore

1. Distinct stratification was absent. A temperature gradient established in the early part of the season had disappeared by July. The annual heat budget was 16,110 g-cal/cm^2, a value of doubtful significance in a lake which is too shallow to store the heat climatically possible. The average transparency was 4.4 m.

2. Many of the chemical conditions were nearly uniform in the lake during the year except for a period in June: dissolved oxygen was abundant at most times, the pH varied little from 9.9, bicarbonate was high except in May when it dropped to zero, carbonate was high at all times, and the phosphate concentration was very high in spring but declined to zero in May and fluctuated erratically throughout the summer. A mineral analysis of the lake water revealed a high content of the sodium salts of carbonate, bicarbonate, sulfate, and chloride, and a total dissolved solids of approximately 14,000 mg/liter.

3. The phytoplankton population was taxonomically simple in that only 18 species and 2 miscellaneous groups were present. The seasonal fluctuations of the chlorophyll concentration, cell number, and cell volume were similar, consisting of early spring and late summer blooms of equal magnitude and midsummer and winter minima.

4. Only the diatoms were of quantitative importance. *Amphora* sp. made up the spring bloom, and *Chaetoceros elmorei* made up the late summer bloom. There was some evidence to suggest that *Amphora* has an optimal temperature range of 8–10°C.

5. Vertical stratification of the phytoplankton was seldom observed. The statospores of *Chaetoceros* were usually found in the bottom waters, indicating a rapid settling rate.

Soap Lake

1. Soap Lake is a meromictic lake with a mixolimnion of greater than 15 m and a monimolimnion situated at a depth of 20 to 28 m. The chemocline lies between 15 and 20 m.

2. Temperature stratification was marked and dichothermic. Both autumn and spring circulations of the mixolimnion occur in years when the lake freezes over. In the winter of 1950–1951 complete surface freezing did not occur, and circulation took place throughout the entire winter. The annual heat budget was 14,902 g-cal/cm^2, a remarkably low value for a relatively deep unprotected desert lake, especially in contrast with Lake Lenore. The average transparency was 5.7 m.

3. Chemical conditions in the mixolimnion indicated dissolved oxygen to a depth of 14 m, a nearly constant pH of approximately 9.9, high carbonate and bicarbonate content, and a high phosphate content with a winter minimum and a summer maximum. The water also contained large quantities of the sodium salts of sulfate and chloride, and had a total dissolved solids of approximately 35,000 mg/liter. The water of the monimolimnion was especially high in dissolved materials with a total content of about 144,000 mg/liter. Annual records of total dissolved solids indicate a dilution of 20 per cent in the mixolimnion between 1950 and 1954, apparently resulting from increased precipitation and seepage from the Columbia Basin Irrigation Project.

4. The chlorophyll concentration is the only quantitative index available of the phytoplankton. The seasonal variation consisted merely of an increase in the population during the winter and the persistence of a minimum throughout the remainder of the year. *Chaetoceros* was present in bloom proportions in 1954 but was not recorded in 1950, and it appears from this that the maximum salinity tolerated is somewhere between 28,000 and 35,000 mg total dissolved solids/liter, the concentrations measured in these two years, respectively.

The effect of grazing by zooplankton on phytoplankton population density is discussed. Population censuses in both lakes

and experimental evidence for Soap Lake suggest that interpretation of the seasonal fluctuations of phytoplankton involves a control by grazing.

REFERENCES

American Public Health Association. 1946. Standard methods for the examination of water and sewage. 9th ed. New York. 286 pp.

Anderson, G. C., G. W. Comita, and Verna Engstrom-Heg. 1955. A note on the phytoplankton-zooplankton relationships in two lakes in Washington. Ecology, **36:** 757–759.

Beadle, L. C. 1943. An ecological survey of some inland saline waters of Algeria. J. Linn. Soc. Zool., **41:** 218–242.

Bretz, J. H. 1932. The Grand Coulee. Amer. Geog. Soc., Spec. Publ. No. 15. 89 pp.

Edmondson, W. T. 1956. Measurements of conductivity of lake water *in situ*. Ecology, **37:** 201–204.

Edmondson, W. T., and Y. H. Edmondson. 1947. Measurements of production in fertilized salt water. J. Mar. Res., **6:** 228–246.

Einsele, W. 1941. Die Umsetzung von zugeführtem, anorganischen Phosphat im eutrophen Seen und ihre Rückwirkungen auf seinen Gesamthaushalt. Z. Fisch., **39:** 407–488.

Hutchinson, G. E. 1937. A contribution to the limnology of arid regions. Trans. Conn. Acad. Arts Sci., **33:** 47–132.

Ketchum, B. H. 1939. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. J. Cell. Comp. Physiol., **13:** 373–381.

Lackey, J. B. 1938. The manipulation and counting of river plankton and changes in some organisms due to formalin preservation. U. S. Pub. Health Rep., **53:** 2080–2093.

Lund, J. W. G. 1950. Studies on *Asterionella formosa* Hass. II. Nutrient depletion and the spring maximum. Part I. Observations on Windermere, Esthwaite Water and Blelham Tarn. Part II. Discussion. J. Ecol., **38:** 1–35.

Mackereth, F. J. 1953. Phosphorus utilization by *Asterionella formosa* Hass. J. Exp. Bot., **4:** 296–313.

Mundorff, M. J., and G. L. Bodhaine. 1954. Investigation of the rise in level of Soap Lake at Soap Lake, Washington. Open-file report of the Water Resources Division, U. S. Geological Survey, Tacoma District, 117 pp.

Rawson, D. S., and J. E. Moore. 1944. The saline lakes of Saskatchewan. Can. J. Res., **22:** 141–201.

Robinson, R. J., and T. G. Thompson. 1948. The determination of phosphates in sea water. J. Mar. Res., **7:** 33–41.

Scheffer, T. H. 1946. Aquatic plants of the Grand Coulee, Washington. Arboretum Bull., Arboretum Foundation, Seattle, Washington. 4 pp.

Scott, G. T. 1945. The mineral composition of phosphate deficient cells of *Chorella pyrenoidosa* during the restoration of phosphate. J. Cell. Comp. Physiol., **26:** 35–42.

Thompson, T. G. 1932. The physical properties of sea water. Bull. Nat. Res. Council, No. 85, pp. 63–94.

Yentsch, C. S., and J. H. Ryther. 1957. Short-term variations in phytoplankton chlorophyll and their significance. Limnol. & Oceanogr., **2:** 140–142.

Young, R. T. 1924. The life of Devils Lake, North Dakota. Pub. North Dakota Biol. Station, 116 pp.

Zwicker, B. M. G., and R. J. Robinson. 1944. The photometric determination of nitrate in sea water with a strychnidine reagent. J. Mar. Res., **4:** 214–232.

Notes on the Precision of Numerical and Volumetric Plankton Estimates from Small-sample Concentrates[1]

JOSEPH H. KUTKUHN[2]

Department of Zoology and Entomology, Iowa State College, Ames, Iowa

ABSTRACT

Standard enumeration and volumetric determination techniques were employed to measure littoral plankton in a shallow prairie lake. Numerical and volumetric estimates were obtained from two-liter water samples which had their plankton (and tripton) content concentrated by means of a continuous-flow centrifuge.

Microplankton were enumerated, and their volumes were calculated on a species and total basis employing a two-stage sampling scheme. Distributions of various species in Sedgwick-Rafter cell mounts from four representative samples (containing at least 73 species) were examined. Occurrences of certain forms in the "fields" of Sedgwick-Rafter cell mounts conformed very closely to the Poisson series while others departed significantly. A majority of the latter were adequately fitted by the negative binomial distribution. For illustrative purposes, totals of selected species distributed as Poisson and negative binomial were estimated and assigned confidence limits. The value of the contagion or over-dispersion factor, $1/\hat{k}$, in constructing confidence limits was pointed out. In addition, the sample size required to yield an estimate having a specified precision was computed for a selected species distributed as Poisson. It was generally concluded that the error of estimated individual species totals usually greatly exceeds the desired level. Hence the size of subsample necessary to secure reasonably precise estimates for the numbers of *each* microplankton species in a given sample concentrate would be, as a rule, quite unwieldy.

Total microplankton counts per subsample were assumed to be normally distributed. Applications of standard statistical techniques indicated that efficiency in the two-stage sampling scheme is most effectively increased by including counts from additional primary sampling units (Sedgwick-Rafter cell mounts). The most practical subsampling ratio from the standpoint of increasing the precision of over-all numerical estimates without expending additional time and labor appeared to be four cell mounts and ten micrometer "fields" per mount.

Microplankton volumetric determinations were made employing a "calculated" volume method. The precision of volumetric unit counts for individual species was indeterminable because of the unknown distribution of the volumetric units. A lack of knowledge concerning such contagious distributions precludes a rigorous assessment of the "calculated" volume's utility.

Macroplankton were counted and had their volume calculated in a manner similar to that described for the microplankton except that the elementary sampling unit constituted an entire Sedgwick-Rafter cell mount. Very adequate numerical and volumetric estimates of individual macroplankton species and total macroplankton were secured from counts and measurements in only three cell mounts per sample concentrate. It was determined that had the allowable error of estimate been relaxed to 20 per cent (±) of the true value, on the average, a sample size of only one cell mount would have sufficed for estimating total macroplankton.

INTRODUCTION

The quantitative assessment of marine and fresh-water plankton populations presents many difficulties, which have received considerable attention since the inception of plankton research. Any biological population with as complex a structure as that of a mixed plankton population is difficult to evaluate. Several studies (*e.g.*, Littleford *et al.* 1940, Gilbert 1942, Serfling 1947, and

[1] Journal Paper No. J-3250 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1374. Iowa Cooperative Fisheries Research Unit sponsored by the Iowa State Conservation Commission and Iowa State College with the cooperation of the U. S. Fish and Wildlife Service.

[2] Now Fisheries Biologist, California Dept. of Fish and Game, Museum Bldg., Stanford University, Palo Alto, Calfornia.

Ballentine 1953) have shown that mechanical errors or those errors arising from manipulation of sample material can, in many cases, be removed or at least reduced by improving collection and preparation techniques. Sampling errors and random variation due to the distribution of the plankters in prepared mounts can often be accounted for by measuring the precision of sample estimates and, if necessary, using this information to modify counting techniques through changes in sampling design.

The present paper represents an attempt to evaluate the plankton techniques employed in a recent study concerned with numerical and volumetric measurement of littoral plankton populations (Kutkuhn 1956). Due to its great volume, it was not practical to subject the sample data to complete statistical treatment. However, to give some insight as to the over-all reliability of the estimates, data from four series of plankton samples chosen as being representative of the type examined during the study were subjected to extensive statistical analysis. The results of this analysis presented herein not only serve to place the findings of the parent study on a firmer basis, but may also have some value as a guide for future plankton studies.

The author extends sincere gratitude to Dr. K. D. Carlander, Iowa State College, for his many helpful suggestions and numerous courtesies. Special thanks are due Drs. J. D. Dodd, Iowa State College, and W. T. Edmondson, University of Washington, for verifying identifications of certain plankton organisms, and Prof. P. G. Homeyer, Iowa State College Statistical Laboratory, for critically reading an early draft of the manuscript.

COLLECTION, CONCENTRATION, AND PREPARATION OF SAMPLE MATERIAL

Water samples for plankton analyses (Table 1) were secured by submerging a two and one-half liter wide-mouth bottle until filled to the two-liter mark. Because of time limitations only a single sample was taken during each sampling period. Thus it was necessary to assume that, for all practical purposes, a single sample contained a reasonable representation of the plankton populations actually occurring at the sampling station during the sampling period.

TABLE 1. *Collection data for the four plankton samples selected for statistical analysis*

Each consisted of a two-liter water sample taken from the littoral zone of North Twin Lake, Iowa, August 1955

Sample number	I	II	III	IV
Date collected	8–2–55	8–2–55	8–2–55	8–4–55
Time collected	8 a.m.	2 p.m.	8 p.m.	2 a.m.
Water temperature	84°F	85°F	84°F	83°F
Air temperature	81°F	85°F	83°F	74°F
Water depth	2 ft.	1 ft.	3 ft.	2½ ft.
Sample depth	½ ft.	½ ft.	½ ft.	½ ft.
Location	East shore	East shore	West shore	West shore
Wind	S:0–3 mph	SSW:0–3 mph	SSE:0–5 mph	Calm

Immediately following its removal from the lake and prior to fixation, the plankton in each water sample was concentrated to about 15 milliliters using a Foerst continuous-flow centrifuge (1955 model: about 20,000 rpm). The centrifuging rate was standardized at about one liter per eight minutes. Very satisfactory fixation was obtained for practically all plankters by flooding the concentrate with 5 ml of very hot 20% formalin solution.

When compared with the plankton net and sedimentation methods, centrifuging was found to be the most rapid and efficient means for concentrating even the smallest nannoplankton. Possible errors in estimates arising from the loss of unusually buoyant forms were assumed negligible since frequent re-centrifugations revealed the loss of such forms during initial centrifuging to be minimal. These extra-buoyant forms were mainly certain blue-green algae (*e.g.*, *Microcystis aeruginosa*) which occurred in relatively small amounts in most samples. Losses of sample material through adhesion to apparatus were minimized by consistently using clean glassware. Undue aggregation caused by compaction during the centrifuging process was never a serious problem. Such a condition could usually be remedied by adding a small quantity of weak detergent solution and properly diluting the concentrate.

Following dilution to the desired degree, the raw concentrate was thoroughly mixed by vigorous agitation. During the mixing process, a one milliliter portion was withdrawn with a large-bore pipette and introduced into a standard Sedgwick-Rafter type counting cell. A cover slip lying diagonally across the cell was permitted to move into place during the operation. Enumeration and volumetric measurement proceeded after the material to be examined had settled in the cell.

All plankton species were grouped for analysis into "microplankton" or "macroplankton" on the basis of relative size. The microplankton included all but a few phytoplankton and the protozoan zooplankton. Several euglenoids and the larger zooplankton comprised the macroplankton.

ENUMERATION OF INDIVIDUAL MICROPLANKTON SPECIES

Microplankton counts were made at a magnification of 210 × (10 × ocular and 8 mm objective). A Whipple ocular micrometer was employed at all times to delimit the "fields" within the Sedgwick-Rafter cell. It was calibrated so that each field had an area of exactly 0.25 square millimeter on the cell's bottom.

Throughout the study all organisms were identified to species, if possible. The 210 × magnification greatly facilitated identification and counting of even very minute plankton. It should be pointed out, however, that all plankton forms were carefully identified and studied (at higher magnification if necessary) prior to quantitative analysis.

The counting technique employed for microplankton (primarily phytoplankton) is that commonly regarded as standard by most planktologists. It is basically a two-stage sampling scheme which involves "random" sampling of Sedgwick-Rafter cell mounts (the primary sampling units) from the thoroughly mixed concentrate, and of 0.25 square millimeter fields (the elementary units) from the cell mounts. The following notation, given here to describe the sampling method, is employed throughout the present discussion:

M = total number of primary sampling units in the original concentrate,
m = number of primary sampling units (Sedgwick-Rafter cell mounts) sampled at "random" from the sample concentrate,
N = total number of elementary units ("fields") contained in each primary sampling unit,
n = number of elementary units sampled at "random" within each primary unit,

where, in samples I, II, III, and IV (Table 1), M = 40, 80, 50, and 50, respectively; m = 2; N = 4,000; and n = 20. Factors or multipliers such as described by American Public Health Association (1955) were calculated for each sample concentrate to facilitate calculation of the total numbers of each plankton species. For microplankton counts, the factor took the form, NM/nm. The product of the calculated factor and the total sample count of a given species gave an estimated total for that species in each two-liter sample. Of primary consideration herein is the question of just how precise were such estimates, precision being defined as the difference between the sample result and the result of a complete count under the same conditions.

To measure the precision with which point estimates are made, it is necessary to have some knowledge of the parent population distribution. It has been generally established that the occurrences of a given kind of plankter in the sampled fields of a Sedgwick-Rafter or other type counting cell are often distributed in Poisson or some related fashion. In plankton enumeration, use of the Poisson distribution implies that the probability with which a randomly distributed organism occurs in any field sampled in the cell mount, although very small, is uniform for all fields. Furthermore, it is obvious that the occurrence of an individual plankter in a field is a function of the plankter's density in the sample concentrate and of the size of the field constituting the subsampling unit.

Many studies, including those of Littleford *et al.* (1940), Gilbert (1942), and Ballentine (1953), have assumed the Poisson

distribution for occurrences of all plankton organisms in prepared mounts. It is of interest to note, however, that most were primarily concerned with estimating total plankton *per se* and did not consider to any extent the effects differences in distributional characteristics of the various species in a given sample might have on the precision of such estimates. In this connection, Serfling (1947) showed that while certain plankters (genera) tended to distribute themselves as Poisson, others failed to do so. In an attempt to account for variation in a plankter's density, he developed criteria for establishing field size on a sample basis so that occurrences of all plankters could be made to follow the Poisson distribution.

Since the present study was concerned not so much with total plankter numbers in each sample concentrate as with individual species totals (and percentage contributions), it seemed advisable to examine each species distribution prior to assessing an estimate's precision rather than to assume the Poisson for all.

In the four samples analyzed 73 different microplankters were identified, counted, and measured. The unit counted and measured constituted the entire organism whether it was a colonial or single-celled form. Interactions between organisms when preparing cell mounts were assumed to be negligible throughout the study.

To check the conformity or non-conformity of sample distributions with the Poisson, the combined occurrences of each microplankton species common to two samples (I and III), and occurring more than once in at least one of the fields counted,[3] were fitted to the Poisson distribution (Table 2). The ratio $\frac{s^2}{\bar{x}}$ was employed to determine whether or not the Poisson could be specified for the sample distributions. Assuming random distribution and selection of counted material, the hypothesis of satisfactory fit was considered not false if the ratio did not exceed 1.3. If the hypothesis were true, $\bar{x}$ became the expected value of σ^2, and the ratio was then distributed as $\frac{\chi^2}{n-1}$ with $n-1$ degrees of freedom. In the present study $\frac{\chi^{2.05}}{n-1}$ (d.f. $= 79$) $= \frac{100.5}{79} = 1.3$ approximately. Hence any value for $\frac{s^2}{\bar{x}}$ exceeding 1.3 indicated rejection of the hypothesis at the .05 probability level. Of the 29 species distributions tested, the hypothesis of satisfactory fit with the Poisson was rejected for 13 (Table 2).

[3] Sixty-seven forms were identified and counted in the two samples. Of these, 21 were not common to both samples, and 17 never occurred more than once in any field counted.

TABLE 2. *Tests of fit of Poisson distribution to counts of 29 microplankton species, and contagion factors ($1/\hat{k}$) for negative binomial distribution*

Counts made in 80 one-fourth square millimeter fields sampled in four Sedgwick-Rafter cell mounts from two two-liter sample concentrates. (Two cell mounts per sample, 20 fields per mount.)

Species	Count	$s^2/\bar{x}$	$1/\hat{k}$
Ankistrodesmus falcatus	43	1.6*	1.39
Aphanocapsa delicatissima	7370	9.3*	0.09
Aphanocapsa pulchra	26	1.2	0.70
Chroococcus limneticus	30	1.2	0.46
Coelastrum microporum	65	1.1	0.11
Cymbella spp.[1]	5	1.4*	5.27
Euglena gracilis	41	0.9	Neg.
Glenodinium quadridens	74	1.4*	0.56
Golenkinia radiata	26	1.4*	0.96
Hastatella radians	13	1.2	0.94
Heterophrys glabrescens	13	1.3	1.92
Kirchneriella lunaris	5	1.4*	5.27
Lagerheimia longiseta	13	3.0*	16.95
Lepocinclis ovum	10	1.3*	2.96
Melosira spp.[2]	228	0.7	Neg.
Merismopedia tenuissima	152	1.5*	0.25
Microcystis aeruginosa	8	1.2	1.60
Navicula spp.[3]	73	2.4*	1.79
Oocystis parva	70	1.8*	1.39
Pediastrum boryanum	36	1.1	0.27
Pediastrum duplex	24	1.0	Neg.
Scenedesmus bijuga	22	2.0*	2.83
Scenedesmus dimorphus	117	1.3	0.21
Scenedesmus spp.[4]	292	1.1	0.04
Schroederia setigera	20	1.4*	2.28
Stephanodiscus niagarae	20	0.9	Neg.
Synedra spp.[5]	800	1.2	0.02
Tetraëdron limneticum	16	1.2	0.95
Tetraëdron regulare	16	0.9	Neg.

* Reject hypothesis (.05 probability level).

[1] Mainly *C. ehrenbergii* and *C. turgidula.*

[2] *M. granulata* and *M.g.* var. *angustissima.*

[3] Mainly *N. bicapitellata*, *N. cryptocephala*, and *N. cuspidata.*

[4] *S. abundans* and *S. quadricauda.*

[5] *S. acus*, *S. ozyrhynchus*, and *S. ulna.*

The problem of over-dispersion (*i.e.*, variance significantly larger than the mean) has recently been reviewed by Bliss and Fisher (1953) in the case of occurrences in nature of plants and animals in general, and has been considered by Holmes and Widrig (1956) in the case of marine phytoplankton enumeration in sedimented concentrates. Particular attention was given the wide applicability and facility of the negative binomial, $(q - p)^{-k}$, where $p = m/k$ and $q = 1 + p$. Expansion of this expression gives the two-parameter (m and k) frequency function

$$f_{(x)} = \frac{(k + x - 1)!}{x!(k - 1)!}\left(\frac{m}{k + m}\right)^x q^{-k}$$

$$x = 0, 1, 2, 3, \cdots$$

where $m/(k + m) = p/q$. The negative binomial is actually a special case of the Poisson, $f_{(x)} = \frac{e^{-m}m^x}{x!}$, wherein the latter's parameter, $m \doteq \bar{x}$, varies continuously in a distribution proportional to that of χ^2. An important feature of the negative binomial is that as the parameter $k \to \infty$, the distribution itself tends toward the Poisson. This is readily seen by noting that as k in the distribution's variance, $\sigma^2 = m + \frac{m^2}{k}$, increases in size, the second term approaches 0 and m approaches σ^2. The value $1/k \doteq 1/\hat{k}$ then represents an index to the degree of over-dispersion or, as used by Holmes and Widrig (1956), to the degree of contagion or "clumping" of plankters in sample concentrates. The larger the value for $1/\hat{k}$, the greater the over-dispersion in the sample distribution.

To ascertain if the over-dispersion exhibited by those species that departed significantly from the Poisson could be accounted for by the negative binomial, the occurrences of 12 of the 13 species in question were fitted to the latter distribution (Table 3). It should be pointed out that k in the negative binomial frequency function was estimated in all cases with at least 90 per cent efficiency, using the moments and maximum-likelihood methods described by Bliss and Fisher (1953). Very close fits were obtained for all species except *Merismopedia tenuissima* and *Oocystis parva*. Failure of these forms to conform with the Poisson or negative binomial could conceivably be attributed to non-randomness of individuals in cell mounts. On the other hand, if the possibility of non-randomness is discounted, the data indicate that the assumption of a Poisson or negative binomial distribution of occurrences for *all* species in sample concentrates may not always be valid. Generally speaking, however, distributions of those forms assumed to be Poisson on the basis of the test described earlier are actually better fitted by the negative binomial if they exhibit any over-dispersion (cf. *Lepocinclis ovum* in Tables 2 and 3). Holmes and Widrig (1956) assumed departure from the Poisson series when $1/\hat{k}$ exceeded 0.05.

PRECISION OF INDIVIDUAL SPECIES ESTIMATES

Once the frequency function is established for a particular species, it is a simple matter to attach confidence limits to its estimated total. Ricker (1937) has compiled tables of .95 and .99 confidence limits for the Poisson distribution, which can be readily applied to plankton estimates. However, these tables give accurate limits only where over-dispersion is negligible, *i.e.*, where $1/\hat{k}$ approaches 0. In actual practice, chances are that a majority of species (including many presumed to be distributed as Poisson) will display some over-dispersion (Table 2). By employing a transformation of the form $y = \log_{10}(x + \hat{k}/2)$, sample data distributed according to the negative binomial can be transformed so as to be nearly normally distributed (Anscombe 1949). Confidence limits can then be constructed for estimates from such a distribution regardless of the degree of over-dispersion exhibited Holmes and Widrig (1956) have constructed graphs of limits of expectation for Poisson and negative binomial distributions having $1/\hat{k} = 0$ ($\hat{k} \neq 0$), 0.2, and 0.5.

For comparative purposes Table 4 gives the estimated numbers and .95 confidence intervals for three microplankters distributed as Poisson $\left[\frac{\chi^2_{.05}}{n - 1}\right.$ (d.f. = 39) =

TABLE 3. *Negative binomial distribution fitted to counts of 12 microplankton species which departed significantly from the Poisson series*

Counts made in 80 one-fourth square millimeter fields sampled in four Sedgwick-Rafter cell mounts from two two-liter sample concentrates. (Two cell mounts per sample, 20 fields per mount.)

Species		Number of fields containing specified number of individuals							χ^2	Eff. d.f.	$\hat{k}$
		0	1	2	3	4	5	6+			
Ankistrodesmus falcatus	Observed	54	15	6	4	1	0	0			
	Expected	53.5	16.5	6.1	2.4	1.5			1.497	2	0.721
Cymbella spp. and *Kirchneriella lunaris*	Observed	76	3	1	0						
	Expected	75.9	3.5	0.6					0.328	1	0.190
Glenodinium quadridens	Observed	39	19	14	7	0	0	1			
	Expected	38.0	23.1	11.0	4.7	3.2			3.211	2	1.781
Golenkinia radiata	Observed	60	16	3	0	1	0				
	Expected	60.3	14.9	3.6	1.2				0.215	1	1.044
Lagerheimia longiseta	Observed	74	3	1	1	0	1	0			
	Expected	73.9	3.2	2.9					0.027	1	0.059
Lepocinclis ovum	Observed	72	6	2	0						
	Expected	71.9	6.6	1.5					0.214	1	0.338
Merismopedia tenuissima	Observed	27	6	18	11	13	5	0			
	Expected	16.9	21.3	16.8	10.6	5.8	8.6		27.410*	3	4.011
Navicula spp.	Observed	47	15	7	6	2	2	1			
	Expected	46.6	16.1	7.8	4.1	2.3	1.3	1.8	1.047	4	0.588
Oocystis parva	Observed	47	11	12	5	5	0	0			
	Expected	46.4	18.3	8.6	4.3	2.4			7.189*	2	0.721
Scenedesmus bijuga	Observed	65	12	1	1	0	1	0			
	Expected	65.3	10.1	4.6					0.934	1	0.353
Schroederia setigera	Observed	66	8	6	0						
	Expected	65.6	10.5	2.7	1.2				3.841	1	0.439

* Statistically significant at the .05 probability level.

1.4], disregarding the contagion factor and taking contagion into account ($1/\hat{k} > 0$). The effect of even a small amount of overdispersion on the size of the confidence interval is obvious.

For practical purposes the use of Ricker's (1937) table in cases where the Poisson is specified seems sufficient, since the efficient estimation of k is often quite laborious. However, it was noted in the present study that in most cases where the Poisson was specified, the nature of k was such that it could be efficiently estimated by the simplest method (method of moments). On the other hand, in those instances where the negative binomial was specified over the Poisson, estimation of k (maximum-likelihood method) became very tedious. In cases where the observed distribution departs significantly from the Poisson and at the same time exhibits a high degree of contagion, a measure of an estimate's precision is best obtained by transforming the sample counts to the normal distribution as noted earlier, calculating the confidence limits, and then re-transforming the data.

Since the theoretical frequency function for occurrences of *Aphanocapsa delicatissima* could not be specified, the question arose as to whether confidence limits could have

TABLE 4. *Confidence intervals (.95) for three microplankton species assumed to be distributed as Poisson, but showing the effect of considering the contagion factor*

Intervals given as thousands of individuals.

Species	Sample	$s^2/\bar{x}$	Disregarding contagion factor	Contagion factor $1/\hat{k}$	Considering contagion factor
Chroococcus limneticus	I	1.3	37–104	0.64	8–440
Glenodinium quadridens	I	0.9	43–119	0.21	24–204
Scenedesmus dimorphus	III	1.4	203–349	0.29	80–850

been placed on estimates of its numbers by employing some "approximation." Perhaps in cases such as the one in point where a species is represented by relatively large numbers, the normal distribution can be used with a fair degree of confidence. An implicit assumption then would be independence of mean and variance, a transformation of some sort being necessary if, in a range of samples having similar composition, the assumption appears invalid.

INCREASING THE PRECISION OF INDIVIDUAL SPECIES ESTIMATES

Sample sizes required to attain a specified degree of precision can be calculated for those species having a known distribution. For those possessing unknown distributions, approximations assuming a normal distribution might be risked. For purposes of illustration, a sample size calculation involving a species with distribution specified as Poisson may be of interest here.

As given by Hansen *et al.* (1953, p. 128), the routine statistical procedure for determining sample size when the sampling fraction (mn/N) is negligible is

$$n_0 = \frac{k^2 V^2}{D^2}$$

where D = the relative error in terms of percentage deviation ($\pm$) from the mean that one is willing to accept; k = the normal deviate introduced to supply a confidence probability level for D; and V = the coefficient of variation.

Now consider *Scenedesmus dimorphus*, distributed as Poisson in sample III (Table 4). Its total count in 40 fields (two cell mounts) was 54. Hence, assuming that the 40 fields constituted a single sample from a population of field counts of *S. dimorphus* having $\bar{x}$ and $s^2 = 54$, and further assuming that such Poisson populations are normally distributed, then the sample size needed to obtain an estimate within 10 per cent of the true value, on the average, 19 times in 20 would be

$$n_0 = \frac{2^2(.136)^2}{.10^2} = 8.$$

Twice this number gives the approximate number of cell mounts in which 20 fields would have had to be counted under the same conditions to attain the specified precision. Admittedly this is a rough approximation (the contagion factor is disregarded) but it should indicate the degree of precision one can expect when making such estimates.

ENUMERATION OF TOTAL MICROPLANKTON

In general, total microplankton counts are more amenable to statistical treatment than counts of an individual species. Due to the large numbers of organisms usually occurring in a sample of fields from a cell mount, the distribution of total microplankton can be assumed to be approximately normal. This assumption is further implemented by the fact that Poisson and related populations having large means are themselves normally distributed. In addition it should be noted that although the sums of variates originating from Poisson populations are themselves distributed as Poisson (Gilbert 1942), it cannot always be shown that all species in a given sample concentrate are distributed accordingly. It follows therefore that the use of the Poisson distribution in the treatment of total plankton counts seems to be rarely justified, hence the reliance on the normal frequency function to approximate what is presumed to be the actual frequency distribution.

Proceeding on this basis, analyses of variance of total microplankton counts indicated that the validity of such counts varies considerably from one sample to the next (Table 5). Differences between cell counts in two of the four samples were highly significant. The mean variation due

TABLE 5. *Variance analyses of total microplankton counts and percentage increase in relative efficiency employing different subsampling ratios for four two-liter water samples*

Sample number		I	II	III	IV	Est. of expected mean square
Source of variation	d.f.	Mean Sq.	Mean Sq.	Mean Sq.	Mean Sq.	
Between cells	1	13,249.6**	1,904.4	878.3	18,105.1**	s_b^2
Between fields within cells	38	386.8	937.6	869.5	1,129.7	s_w^2
Total	39					
Variance ($\bar{\bar{x}}$), original sample		331.2	47.6	21.9	452.6	
Percentage increase in efficiency for:						
1. $m = 3$ $n = 10$		47	0	−25	52	
2. $m = 3$ $n = 20$		50	50	51	50	
3. $m = 4$ $n = 10$		94	34	0	88	
4. $m = 4$ $n = 20$		100	100	99	100	
5. $m = 6$ $n = 20$		200	200	200	200	
6. $m = 6$ $n = 10$		191	102	50	183	

** Statistically significant at .01 probability level.

to differences between cell counts was also significant, as was determined from the combined analysis of variance ("F" = 10.270, d.f. = 4 and 152). Thus there appears to be little assurance that the validity of counts in one sample employing a particular counting scheme would be duplicated in the next employing the same method. Total microplankton estimates from samples II and III were in error by less than 10 per cent of the mean, whereas those of samples I and IV barely fell within the 20 per cent range of relative error (Table 6). It appears then that the logical objective when analyzing a series of plankton samples would be, if possible, to reduce the sampling error in those samples exhibiting the highest amount of variation and at the same time proportionately increase the precision of counts in the remaining samples even though they may already fall within prescribed limits of error.

The change in sampling scheme that would have resulted in the desired reduction of the over-all sampling error could have been easily determined by routine statistical methods. Cochran (1953, p. 219) describes a method for predicting mean variances in the two-stage sampling design employing different subsampling ratios. An estimate of the over-all true mean variance ($\sigma^2_{\bar{\bar{x}}}$) is given by

$$s^2 = \frac{s_c^2}{m} + \frac{s_w^2}{mn}$$

where, from the analysis of variance of each sample (Table 5),

$$s_c^2 = \frac{s_b^2 - s_w^2}{n}.$$

By substituting appropriate values in the denominator, the mean variance for any combination of cell mounts and fields can be readily determined. It is apparent that an increase in the number of fields counted reduces only the among-fields variance (s_w^2), whereas an increase in the number of cell mounts sampled from the concentrate decreases both components of the over-all variance. From the standpoint of reducing the over-all mean variance, it is further apparent that had the number of fields counted per cell mount remained the same,

counts from an additional mount would have increased the precision of the over-all total estimate by about 50 per cent (Table 5). Increasing the number of cell mounts by one and decreasing the number of fields per mount by one-half would have resulted in increased precision for estimates from samples I and IV but not for estimates from samples II and III.

The extent to which estimates from field counts in a single Sedgwick-Rafter cell mount might be considered valid was investigated to obtain some idea of the number of fields in any given cell mount required to attain a specified precision. The results (Table 7) verify the point made earlier that the major source of variation in two-stage sampling can generally be expected to arise from differences between cell counts. The choice of 20 fields as the sub-sample size appears to have been a good one, yielding individual cell estimates in error, on the average, by only about 10 per cent. Though total microplankton estimates from single cell mounts using 20 fields would have been fairly reliable, it is evident that the large amount of variation attributable to differences between counts in successive cell mounts might often tend to nullify even the most precise estimates from individual cell mounts.

In the present study, counts from an additional cell mount would have resulted in increased precision (about 50 per cent), but they would also have necessitated the expenditure of considerable extra time and labor. By using a subsampling scheme wherein 10 fields are counted in each of four cell mounts, over-all estimates having a net increase in precision (to within 10–12 per

TABLE 6. *Estimated number of microplankton organisms and estimated volume of microplankton, macroplankton, and tripton*[1] *(in cubic millimeters) in each of four two-liter water samples, North Twin Lake, August, 1955*

(The numbers following ± indicate 0.95 confidence intervals assuming normal distributions.)

Sample	I	II	III	IV
Number of microplankton organisms (in thousands)	17,088 ± 5,824	54,496 ± 4,416	27,460 ± 1,880	28,880 ± 8,520
Microplankton volume	18.70 ± 1.2	38.94 ± 2.7	24.36 ± 5.4	24.46 ± 3.7
Macroplankton volume	2.56 ± 0.2	1.38 ± 0.2	2.26 ± 0.4	2.71 ± 0.4
Tripton[1]	19	41	33	19

[1] Included for rough comparative purposes only; estimates necessarily based upon crude volumetric unit determinations.

TABLE 7. *Numbers of one-fourth square millimeter fields per Sedgwick-Rafter cell mount needed to attain specified precision in estimating total microplankton*

Sample number	I		II		III		IV		
Cell number	1	2	1	2	1	2	1	2	
Total organisms	1772	2500	3544	3268	2840	2653	2461	3312	
Mean	88.6	125.0	177.2	163.4	142.0	132.6	123.1	165.6	
St. deviation	16.2	22.6	30.4	30.8	35.2	22.4	14.3	45.3	
Coef. of var.	.182	.181	.171	.188	.248	.169	.116	.273	
Coef. of var.[2]	.033	.033	.029	.035	.062	.029	.013	.075	Mean
At .99 probability allowable error:									
8%	45	45	41	49	87	41	18	105	54
At .95 probability allowable error:									
4%	83	83	73	88	155	73	33	188	97
8%	21	21	18	22	39	18	8	47	24
10%	13	13	12	14	25	12	6	30	18
12%	9	9	8	10	18	8	4	21	11
20%	4	4	3	4	7	3	2	8	5

cent of the mean at the 95 per cent confidence probability level) could have been acquired with the same amount of effort as expended for each sample concentrate. On the basis of the foregoing, the superiority of two-stage sampling over "random" sampling of fields from a single cell mount becomes readily apparent.

ESTIMATION OF MICROPLANKTON VOLUME

During the parent study considerable attention was given the problem of ascertaining each plankton species contribution to the total plankton volume in each sample concentrate. The "calculated volume" method introduced by Lohmann (1908) (see Ruttner, 1953, pp. 145–146) represents one of the best methods devised for determining the mass of living organisms and the proportions that individual species contribute to its composition. Thunmark (1945) employed basically the same technique to determine species dominants in net-plankton samples. Nygaard (1949) criticized the method, however, because of its obvious subjectivity. The principal objection was that in measuring a given organism prior to computing its volume, the estimate of its thickness is often subject to considerable error.

Notwithstanding possible subjective errors, the "calculated volume" or "cubic standard" method employed by the above workers and fully described by American Public Health Association (1955) was used throughout the parent study. Briefly, all organisms encountered during the enumeration process were measured with the aid of the ocular micrometer and, where necessary, the calibrated fine adjustment. All volumes were recorded in terms of cubic standard units (one cubic standard unit at 210× magnification = $.01^3$ ml or 1,000 μ^3). Nomographs and tables given by American Public Health Association (1955) aided greatly in calculating volumes of regularly-shaped organisms. In securing estimates of the total number of volumetric units for each species comprising a sample concentrate, further use was made of the factors or multipliers described earlier for the plankton counts. Unfortunately, the assignment of confidence limits to such estimates was precluded due to an inadequate knowledge of the frequency distributions of the volumetric units which make up each plankton species.

While it has been established that most plankters (colonies or noncolonial cells) are distributed in Poisson fashion in the counting chamber, such obviously is not the case for individual cells of colonial forms (Gilbert 1942). If the volumetric units as used herein be considered analogous to individual cells of a colonial plankter, it follows that the product of two frequency distributions is being dealt with, *viz.* a Poisson (or other) distribution of occurrences and an unknown and variable contagious distribution of the number of volumetric units per occurrence. Further complications arise in cases where both distributions are correlated. Such a situation might result from environmental conditions under which an increase in an organism's volume may be related to an increase in population size. Because of the obvious difficulties, the question of how reliable were volumetric estimates at the species level remains in doubt.

The problem of dealing with this type of contagious distribution has been considered by Fisher *et al.* (1943), who studied the relation between the number of species and the number of individuals in a random sample of an animal population. Use was made of the logarithmic distribution which results when k in the negative binomial distribution approaches zero ($1/\hat{k}$ is very large) and the zero-frequency class is omitted. It appears conceivable that application of the logarithmic series to the observed frequencies of volumetric units per individual plankter would be appropriate for educing the desired error measurement. More explicitly, Anscombe (1950) points out that if colonies or groups of individuals are distributed as Poisson, and if individuals in these groups are independently distributed in logarithmic fashion, then the negative binomial distribution applies for the total count of individuals. The main problem in the present case is whether the distributions

of volumetric units comprizing each individual plankter can be assumed independent of the latter's distribution.

Estimates of total microplankton volume per sample concentrate were also of interest from the standpoint of their reliability. Total volumetric unit estimates from three of the four samples considered herein (Table 6) were in error by less than 10 per cent of the mean. A basic assumption here was that the subsample total volumetric counts were normally distributed, as indicated by the apparent independence of mean and variance. This assumption was made with some reservation, however, because of the underlying contagious nature of the volumetric units distribution.

A comparison of total microplankton unit counts revealed that in only one of the four samples were the mean counts from two successive cell mounts significantly different (Table 8). A combined analysis of variance for the four samples disclosed also the average difference between cell counts to be insignificant ("F" = 2.108; d.f. = 4 and 152). It is felt that since there were no significant differences between cell counts, the estimated volumetric unit totals in samples I, II, and IV would have been sufficiently reliable for practical purposes. However, a reduction in sampling error may have been warranted for sample III. Using the method outlined earlier for the treatment of numerical data, it was determined that by increasing the number of cell mounts to three, a 50 per cent increase in precision could have been realized.

If estimates of total volumetric units contained in any one sample concentrate had been obtained from counts in single cell mounts, then counts in an average of over twice as many fields would have been needed

TABLE 8. *Variance analyses of microplankton volumetric unit counts in four two-liter water samples*

Sample number		I	II	III	IV
Source of variation	d.f.	Mean sq.	Mean sq.	Mean sq.	Mean sq.
Between cells	1	616.3	714.1	7,452.9**	3,422.5
Between fields within cells	38	1,410.0	1,232.4	1,274.1	1,892.4
Total	39				
Variance ($\bar{x}$), original sample		15.4	17.9	186.3	85.6

** Statistically significant at .01 probability level.

TABLE 9. *Numbers of one-fourth square millimeter fields per Sedgwick-Rafter cell mount needed to attain specified precision in estimating total microplankton volumetric units*

Sample number	I		II		III		IV		
Cell number	1	2	1	2	1	2	1	2	
Total vol. units	2259	2416	2518	2349	2708	2162	2260	2630	
Mean	112.9	120.8	125.9	117.5	135.4	108.1	113.0	131.5	
St. deviation	27.6	45.1	31.5	38.4	40.1	30.6	44.7	42.3	
Coef. of var.	.244	.373	.250	.327	.296	.283	.396	.322	
Coef. of var.^{2}	.059	.139	.063	.107	.088	.080	.157	.104	Mean
At .99 probability allowable error:									
8%	83	196	89	151	124	113	221	146	141
At .95 probability allowable error:									
4%	148	348	157	268	220	200	393	260	249
8%	37	87	40	67	55	50	98	75	64
10%	24	56	25	43	35	32	63	42	40
12%	17	39	18	30	25	22	44	29	28
20%	6	14	7	11	9	8	16	11	9

TABLE 10. *Counts of nine common macroplankton organisms in each of four two-liter water samples from North Twin Lake, Iowa: August 1955*

Sample number	I			II			III			IV		
Cell number	1	2	3	1	2	3	1	2	3	1	2	3
Protozoa:												
Euglena ehrenbergii	23	28	30	3	9	5	3	4	0	0	1	0
Euglena oxyuris	205	204	238	24	27	25	13	8	8	1	0	3
Rotatoria:												
Anuraeopsis fissa	13	19	24	53	52	71	110	90	124	120	132	134
Brachionus angularis	10	13	11	2	0	3	19	16	17	21	17	39
Brachionus caudatus	2	2	4	0	0	0	5	5	6	4	12	10
Filinia longiseta	81	86	68	27	30	29	61	46	47	58	86	81
Pedalia mira	11	9	10	3	4	2	7	3	4	7	5	7
Polyarthra sp.	202	145	202	11	12	16	90	71	70	94	96	90
Trichocerca spp.[1]	32	51	46	21	32	27	31	27	32	8	12	16
Totals	579	557	633	144	166	178	339	270	308	313	361	380
Sample totals		1769			488			917			1054	

[1] *T. longiseta*, *T. rousseleti*, *T. similis*, and *T. stylata*.

to bring the relative error of such estimates to within 10 per cent of the mean (Table 9). However, a two-stage sampling scheme, differing from that employed by having the number of cell mounts sampled slightly increased and the number of fields counted actually reduced, would have yielded much more reliable estimates of the true sample volume without sacrificing time and effort.

ENUMERATION OF THE MACROPLANKTON

Estimates of total macroplankton (primarily large zooplankton) were obtained from counts in three Sedgwick-Rafter cell mounts from each sample concentrate. The entire contents of each cell mount constituted the sampling unit. All counts were made at a magnification of 100×.

On the basis of the relatively large numbers of macroplankton observed, it was assumed that counts of most macroplankton species were distributed in approximately normal fashion. Actual counts of the nine most common of 22 macroplankters encountered reveal relatively little variation between counts of individual forms in successive cell mounts (Table 10). Such a condition seemed to be typical of macroplankton counts in sample concentrates examined throughout the parent study.

An analysis of variance of the data in Table 10 disclosed differences between

TABLE 11. *Analysis of variance of (transformed) macroplankton counts*

Original data given in Table 10

Source of variation	d.f.	Sum of squares	Mean square	*F*
Between samples	3	134.696	44.898	3.86*
Between cells within samples	8	141.208	17.651	1.52
Between species within cells within samples	96	1,117.914	11.645	
Total	107	1,393.818		

* Statistically significant at .05 probability level.

transformed[4] mean counts in successive cell mounts to be, on the average, statistically insignificant (Table 11). It is also interesting to note that differences between the transformed sample means were barely significant at the .05 probability level, indicating that, on the average, little change in the numbers of total macroplankton occurred during the three-day period over which the four samples were collected. Although appreciable changes in the total macro-

[4] Because of the presence of several zeros in the data, a square-root transformation of the form $\sqrt{x + 0.5}$ was made in an attempt to satisfy the assumption of normality and thus permit use of the "*F*" test.

TABLE 12. *Estimated total macroplankton in each of four two-liter water samples and the number (n_0) of Sedgwick-Rafter (1 ml) cell mounts needed to attain the specified precision*

Sample number.....	I	II	III	IV
Total organisms	1769	488	917	1054
Mean	589.7	162.7	305.7	351.3
St. deviation	39.1	17.2	34.6	34.5
St. error	22.6	10.0	19.9	19.9
.95 conf. interval	589.7 ± 45.2	162.7 ± 20.0	305.7 ± 39.8	351.3 ± 39.8
Estimated total no.	23,588 ± 1,808	13,016± 1,600	15,285 ± 1,990	17,565 ± 1,990
Coef. of var.	.066	.105	.113	.098
Coef. of var.^{2}	.004	.011	.013	.010
At .95 probability allowable error:				
10% n_0	2	4	5	4
20% n_0	1	1	2	1

plankton numbers may not have taken place, it is fairly evident (Table 10) that the numbers of individual species did fluctuate considerably.

Estimates of the total macroplankton numbers in each of the four samples are given in Table 12. On the average, it appears that the relative error of these estimates is somewhere in the vicinity of ±15 per cent of their respective means. If estimates in error by as much as ±20 per cent would have been satisfactory for the purposes of the study, then, on the average, a sample size[5] of only one Sedgwick-Rafter cell mount would have sufficed. However, since macroplankton enumeration is a fairly simple process, extension of the sample size from three to five cell mounts would not create any hardship and would measurably increase the precision of the estimates of most forms.

Volumetric determinations of the various macroplankton species and of the total macroplankton proceeded on the same basis as described earlier for the microplankton with but one exception. Instead of calculating the volume of each organism as it was encountered in the counting process, the total number of each macroplankton species was multiplied by an average total volume for that particular form. Such averages were calculated on the basis of volume measurements of about 50 of each species. It was felt that since most macroplankters were fairly constant in size, such an approach did not detract appreciably from the utility of the "calculated volume" method. Furthermore, the expediency of this modification of the original method is obvious.

It is evident (Table 6) that the contribution of the macroplankton (mainly zooplankton) to the biomass was considerably less by comparison with that of the microplankton (mainly phytoplankton). Such a condition is seemingly typical of highly eutrophic temperate lakes during mid-summer when the phytoplankton (primarily Cyanophyta) maintains a high level of development and the zooplankton drops to its lowest.

[5] It should be noted here that since the sampling fraction (n/N) in three of the four samples exceeded five per cent, it was necessary to introduce the finite correction factor into the formula used previously for calculating sample size. As given by Hansen *et al.* (1953, p. 127) the corrected formula is $n_0 = \frac{k^2NV^2}{ND^2 + k^2V^2}$ where N = the total number of sampling units in the sample concentrate.

SUMMARY

Standard enumeration and volumetric determination techniques were employed in the quantitative assessment of littoral plankton populations. Two-liter water samples constituted the basis for analyses of such populations. Prior to analysis, each sample had its plankton (and tripton) content concentrated to about a hundredth of the original sample size by means of a continuous-flow centrifuge.

Microplankters were enumerated and had their volume calculated on a species and total basis employing a two-stage sampling scheme. Distributions of various species in Sedgwick-Rafter cell mounts from four representative samples were examined. Certain forms conformed very closely to the Poisson distribution while others departed significantly. Distributions of several species were best fitted by the negative binomial frequency function. For illustrative purposes, total estimates of selected species distributed as Poisson and negative binomial were calculated and assigned confidence limits. The value of the over-dispersion or contagion factor, $1/\hat{k}$, in making interval estimates was pointed out. The sample size required to yield estimates having a specified precision was computed for a selected species distributed as Poisson. It was generally concluded that the precision of total estimates of individual species is usually less than the desired level. The size of subsample required to obtain reasonably precise estimates of each microplankton species in a particular sample would be, as a rule, quite unwieldy.

Total microplankton counts per subsample were assumed to be normally distributed. Applications of standard statistical procedures indicated that efficiency in the two-stage sampling scheme is most effectively increased by including counts from additional primary sampling units (Sedwick-Rafter cell mounts). The most practical subsampling ratio from the standpoint of increasing the precision of over-all estimates appeared to be four cell mounts and ten fields per mount.

Microplankton volumetric determinations were made employing a "calculated volume" method. The precision of volumetric unit counts for individual species was not estimated because of the unknown distribution of the volumetric units.

Total microplankton volume determinations were made on the same basis as were total microplankton counts. Total microplankton volumetric unit counts were assumed to be normally distributed. The subsampling ratio as employed (two cell mounts and 20 fields per mount) yielded reasonably precise estimates. Increasing the cell mounts sampled to four and reducing the number of fields per cell mount to ten would have increased the over-all precision of total volume estimates.

Macroplankton on both a species and total basis were estimated numerically and volumetrically. The sampling procedure for macroplankton differed somewhat from that for microplankton in that an entire Sedgwick-Rafter cell mount comprised the sampling unit. Estimates were made from counts of individuals and the number of volumetric units per individual in three cell mounts from each sample concentrate. For all practical purposes, about the same degree of precision as that obtained could have been realized from counts in only one or two cell mounts, although counts from as high as five mounts would have created no hardships from a labor standpoint.

On the basis of the present study, indications are that total microplankton numbers *and* volume could have been estimated with increased precision if slight changes had been made in the sampling design. The two-stage sampling scheme employing four primary sampling units and ten elementary units would have served adequately for both. Such a change might also have resulted in more precise estimates of at least some individual species.

REFERENCES

American Public Health Association. 1955. Standard methods for the examination of water, sewage, and industrial wastes. 10th Ed., New York: A.P.H.A., A.W.W.A., and F.S.I.W.A. 522 pp.

Anscombe, F. J. 1949. The statistical analysis of insect counts based on the negative binomial distribution. Biometrics, **5:** 165–173.

———. 1950. Sampling theory of the negative binomial and logarithmic series distributions. Biometrika, **37:** 358–382.

Ballentine, D. 1953. Comparison of the different methods of estimating nanoplankton. J. Mar. Biol. Assoc. U.K., **32:** 129–148.

Bliss, C. I., and R. A. Fisher. 1953. Fitting the negative binomial distribution to biological data. Note on the efficient fitting of the negative binomial. Biometrics, **9:** 176–200.

Cochran, W. G. 1953. Sampling techniques. J. Wiley & Sons, N. Y. 330 pp.

Fisher, R. A., A. S. Corbett, and C. B. Wil-

LIAMS. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. J. An. Ecol., **12:** 42–58.

GILBERT, J. Y. 1942. The errors of the Sedgwick-Rafter counting chamber in the enumeration of phytoplankton. Trans. Am. Micros. Soc., **61:** 217–226.

HANSEN, M. H., W. N. HURWITZ, AND W. G. MADOW. 1953. Sample survey methods and theory. Vol. 1: Methods and applications. J. Wiley & Sons, N. Y. 638 pp.

HOLMES, R. W., AND T. M. WIDRIG. 1956. The enumeration and collection of marine phytoplankton. J. Cons. Int. Explor. Mer, **22**(1): 21–32.

KUTKUHN, J. H. 1956. Some ecological relationships of the gizzard shad, *Dorosoma cepedianum* (LeSueur), in a dredged prairie lake. Ph.D. thesis, Iowa State College Library, Ames, Iowa. 179 pp.

LITTLEFORD, R. A., C. L. NEWCOMBE, AND B. B. SHEPHERD. 1940. An experimental study of certain quantitative plankton methods. Ecology, **21:** 309–322.

LOHMANN, H. 1908. Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton. Wissensch. Meeresunters., **10:** 131–370.

NYGAARD, G. 1949. Hydrobiological studies in some ponds and lakes. Part III. The quotient hypothesis and some new or little known phytoplankton organisms. Kgl. Danske Vidensk. Selsk. Biol. Skrifter, **7**(1): 1–293.

RICKER, W. E. 1937. The concept of confidence or fiducial limits applied to the Poisson frequency distribution. J. Am. Stat. Assoc., **32:** 349–356.

RUTTNER, F. 1953. Fundamentals of limnology. (Translated from the German by D. G. Frey and F. E. J. Fry.) Univ. Toronto Press, Toronto. 242 pp.

SERFLING, R. E. 1947. Quantitative estimation of plankton from small samples of Sedgewick-Rafter-cell mounts of concentrate samples. Ph.D. thesis, Univ. Mich. Library, Ann Arbor, Mich.

THUNMARK, S. 1945. Zur Soziologie des Süsswasserplanktons. Eine methodologisch-ökologische Studie. Folia Limnol. Scand., **3:** 1–67.

On the Dynamics of Phosphorus Added to Fishponds in Israel[1]

B. Hepher

Fish Culture Research Station, Dor, Israel

ABSTRACT

The fate of phosphate added to fishponds and the inorganic processes of phosphorus fixation were studied in Israel. Conditions in these ponds are different from those of the more humid climatic zones in having alkaline water and high calcium content. In these conditions most of the phosphorus added is precipitated as $Ca_3(PO_4)_2$. The quantitative theoretical aspects of this are discussed. Another factor in the phosphorus fixation is the bottom mud. This fixation in the alkaline conditions prevailing is believed to be partly due to anion exchange reaction of the colloids and partly to reaction with calcium carbonate and not with heavy metals. The fixation of phosphorus to the mud is affected by the composition of the mud, becoming greater when the content of calcium carbonate and total colloids is higher. Fixation of phosphorus is lower with the accumulation of organic matter in the mud because of the low phosphorus adsorption capacity of organic colloids. Phosphorus fixation is higher when the mud is mixed with the water of the pond. In the fishpond this is done by the carp in its search for food in the bottom mud.

INTRODUCTION

Although the manuring of fishponds as a means of increasing fish production was known in China in the fourth and fifth centuries B. C. (Neess 1949), the use of chemical fertilizers for the same purpose has only developed in the last few decades.

The results of many experiments on fishpond fertilization reviewed by Mortimer and Hickling (1954) show that chemical fertilizers can bring about considerable increase in the yield of fish.

Opinions differ regarding the importance of nitrogen and potassium fertilizers, as conflicting experimental results have been obtained. Most workers agree on the importance of phosphorus and its striking effect on fish yield. This effect is no doubt due to the fact that phosphorus is generally found in very low concentrations in fresh water and is a limiting factor in the development of the phytoplankton of the pond.

[1] The following study is a revision of part of a Ph.D. thesis submitted to the senate of the Hebrew University, Jerusalem, July 1956, under the guidance of Prof. S. Ravikovitch of the Faculty of Agriculture, to whom the author is very much indebted. Thanks are also due to Prof. K. Reich of the Department of Zoology for his advice and encouragement, to the Fish Breeders Association in Israel for its help, and to Mr. K. E. Marshall of the Freshwater Biological Association, England, for going over the English translation.

The fertilization of fishponds in Israel with phosphorus was begun in 1946. Since then it has become a general practice in fishculture. Each pond receives approximately 0.7–1.0 tons of superphosphate per hectare per annum. Five regional laboratories situated in different parts of Israel were constantly checking the phosphorus concentration before and after each fertilization. These analyses showed that although considerable amounts of water-soluble phosphorus were added to the water (sufficient to raise the phosphorus concentration to 0.5 mg/L or more), in a short period (24–48 hours) after the fertilization only a small fraction of this phosphorus, usually not more than 1–5 %, remained.

This rapid disappearance of the phosphorus from the water was noted by Reich (1950) who pointed out that this is amazingly rapid because the amounts of phosphorus added are above those needed by the phytoplankton for good growth. Fisher and Rashkes (1951) added phosphorus in very high doses (150–200 kg/ha) compared with those normally given to fishponds. In 31% of the cases they found after a short period only 1% of the amount added. In all cases there was an increase in phosphate concentration of not more than 3–5 % above the control. The rate of disappearance of

phosphorus from the water was not proportional to the amounts added.

This rapid decline in phosphorus concentration after fertilization was also noted by workers outside Israel. Nisbet (1951) and Zeller (1952) give similar results. Breest (1921) found no significant difference in the concentration of phosphorus between fertilized and unfertilized ponds.

Any attempt to determine the optimum quantity of phosphorus fertilizer to be added to the fishponds, the kind of fertilizer to be used, the methods of application, and the time of fertilization should first solve the problem of the fate of the phosphorus added. Is it utilized wholly or only partly by the phytoplankton? If only partly what are the other factors responsible for the rapid decrease of the phosphorus concentration in the water following the fertilization? The present study aims to throw some light on this problem.

METHODS

The experiments described in this paper were conducted mainly in the laboratory although some "field experiments" were carried out in earthen ponds. In the laboratory experiments 16-litre enamel containers or smaller glass vessels were used. The experiments were designed to find the role of soil and its effect on phosphorus concentration in water. A layer some 4–5 cm thick of various soils was put on the bottom of the water-filled vessels. The ponds used for experiments were of B block of the Fish Culture Research Station at Dor and each was 400 m² in area. The laboratory experiments were conducted in quadruplicate and the pond experiments only in triplicate.

Chemical analyses

The water for chemical analyses was sampled so as to give a true composition of the entire water column in the containers and ponds. This was done by using a glass tubing fitted at the bottom with a conical rubber stopper. The rubber stopper was pulled into place by means of a plastic cord passing down the tube after the tube had filled with water. Sampling in the ponds was done with an apparatus described by Wirshubsky (1952) which operates on the same principle (Fig. 1).

Water soluble phosphate was determined by the standard methods of the American Public Health Association (1946) using stannous chloride as a reduction agent. Light transmittance after reduction was determined with the "Coleman Junior" spectrophotometer using a wavelength of 6750 Å.

Total phosphorus was determined by the Robinson method as described by Rochford (1951) using perchloric acid as the oxidizing agent.

Phosphorus bound to the mud particles was determined by a method described by Rochford using perchloric and sulfuric acids for oxidation.

pH was determined either colorimetrically or using a glass-calomel pH-meter.

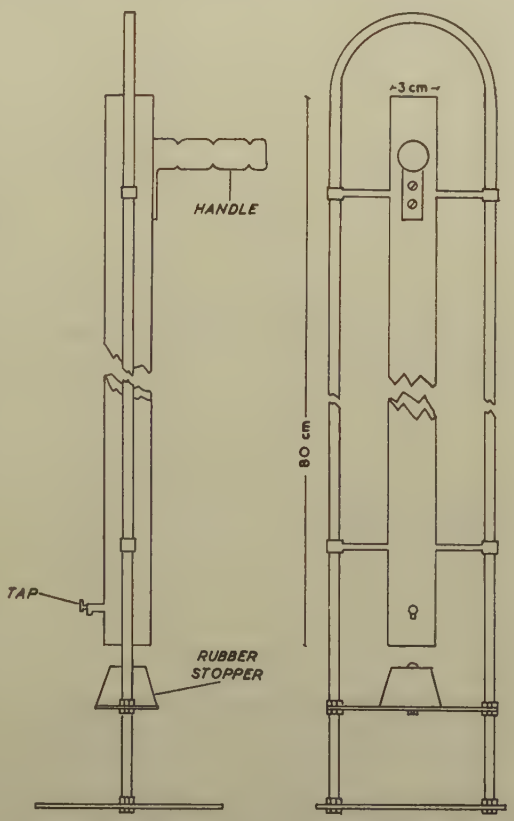

FIG. 1. A water sampler for fishponds (described by Wirshubsky 1952).

Alkalinity was determined as described in the American Public Health Association's Standard Methods (1946), and results were expressed in values of $CaCO_3$ for carbonate alkalinity and bicarbonate alkalinity assuming an ideal system of hydroxide-carbonate-bicarbonate.

Soils

Four different soils were used in the experiments: (1) calcareous clay-loam soil from Messiloth, Beith-Shan valley; (2) alluvial clay soil, poor in calcium from Kfar Jehoshua, Izrael valley; (3) pond bottom mud from a pond at Nir-David, Beith-Shan valley; (4) calcareous clay-loam soil from the bank of the same pond. The composition of these soils is as follows (in per cent of dry weight):

	(1)	(2)	(3)	(4)
Hygroscopic moisture	3.8	10.0	5.9	4.8
Organic matter	3.1	1.74	2.50	1.40
Calcium carbonate	68.8	0.23	32.7	32.9
Total phosphorus (P_2O_5)	0.21	0.126	0.137	—
Textural composition (%)				
Sand	6.19	0.09	8.0	26.18
Fine sand	32.94	22.95		
Silt	38.8	22.93	37.8	32.18
Clay	22.07	54.03	54.2	41.64
pH	7.66	7.5	—	—

Water

The experiments were conducted in two places with slightly different composition of the water. Partial composition of the water used is as follows (in mg/L):

	Hadera tap-water	Dor tap-water
Nitrate nitrogen	3.24	2.30
Ammonia nitrogen	0.1	0.14
Phosphorus (P)	0.054	0.004
Chloride	96.3	353
Calcium	52.0	98.2

Fertilizers

In order to approximate as closely as possible the conditions in the pond following fertilization the fertilizers used in the experiments were not the pure chemicals but rather the usual phosphate fertilizers such as superphosphate. The phosphorus content of the fertilizers was found to be as follows:

Superphosphate (batch A)—18.1 % P_2O_5
Superphosphate (batch B)—14.6 % P_2O_5
Crude phosphoric acid —29.3 % P_2O_5

The amount of fertilizer added was in most cases equivalent to fertilization of 200 kg/ha. This is a large dose, but as shown by Rashkes (1949) this does not affect the rate of disappearance of phosphorus after the fertilization. It was found to be more convenient to give a large dose rather than a smaller one in the small volumes of water used. The calculation of the added dosage was made from the volume of water and not from the surface area as is usual in fish farming practice. One hectare of pond was considered to contain 8000 m^3 of water.

RESULTS

The effect of phosphorus fertilization in raising the fish yield is no doubt due to the assimilation of it by the phytoplankton which is at the base of the food chain. As this study was concerned mainly with the effect of other factors on the disappearance of the water soluble phosphate, phytoplankton was excluded from the experiments by using tap-water which is devoid of phytoplankton. The first set of experiments was concerned with the effect of the soil on the phosphorus concentration in the water. Special attention was given to the effect of some components of the soil such as organic matter, which accumulates on the bottom, and calcium carbonate, which is the main component of the soil in some fish culture regions (*e.g.*, Beith Shan valley and Jordan valley where it sometimes comprises up to 65–70 % of the soils).

Experiment 1

The first experiment consisted of four treatments as follows:

(*A*) Control: Tap water only.
(*B*) Calcareous soil [68.8 % $CaCO_3$. Designated as (1) in the description of soil types used].
(*C*) Soil poor in calcium carbonate [0.23 %. Soil type (2)].
(*C–S*) The same as (*C*), but this soil was sterilized by adding 3 % formalde-

hyde to study the effect of biological fixation of the phosphorus by soil microorganisms.

During the period of the experiment care was taken not to disturb the mud and to keep the water still. Four-hundred mg of powdered superphosphate (ground and mixed before application) were added to each container. This was sufficient to raise the phosphorus concentration to the level of 1.98 mg P/L. The application of the phosphorus was made 72 hours after filling the containers with water so that equilibrium could be reached between soil and water before the addition of phosphate.

The results of this experiment are given in Figure 2. It can be seen that there was a constant decrease in phosphate concentration in the water although it was much less rapid than that seen in the ponds. The maximum concentration is reached only 2–3 days after the application. This is the time taken for the phosphorus to become completely dissolved and distributed in the water.

The phosphorus concentration in the control increased at first, but from the third day it constantly decreased although no soil was present. The treatment with the calcareous soil (*B*) showed an immediate fixation of approximately 24% of the phosphorus by the third day, as compared with the control, and during the period of the experiment no further fixation was noticed. The decrease in phosphorus concentration was roughly parallel to that in the control, remaining at a level of 73.5–77.5% of that of the control. In this case it seems that

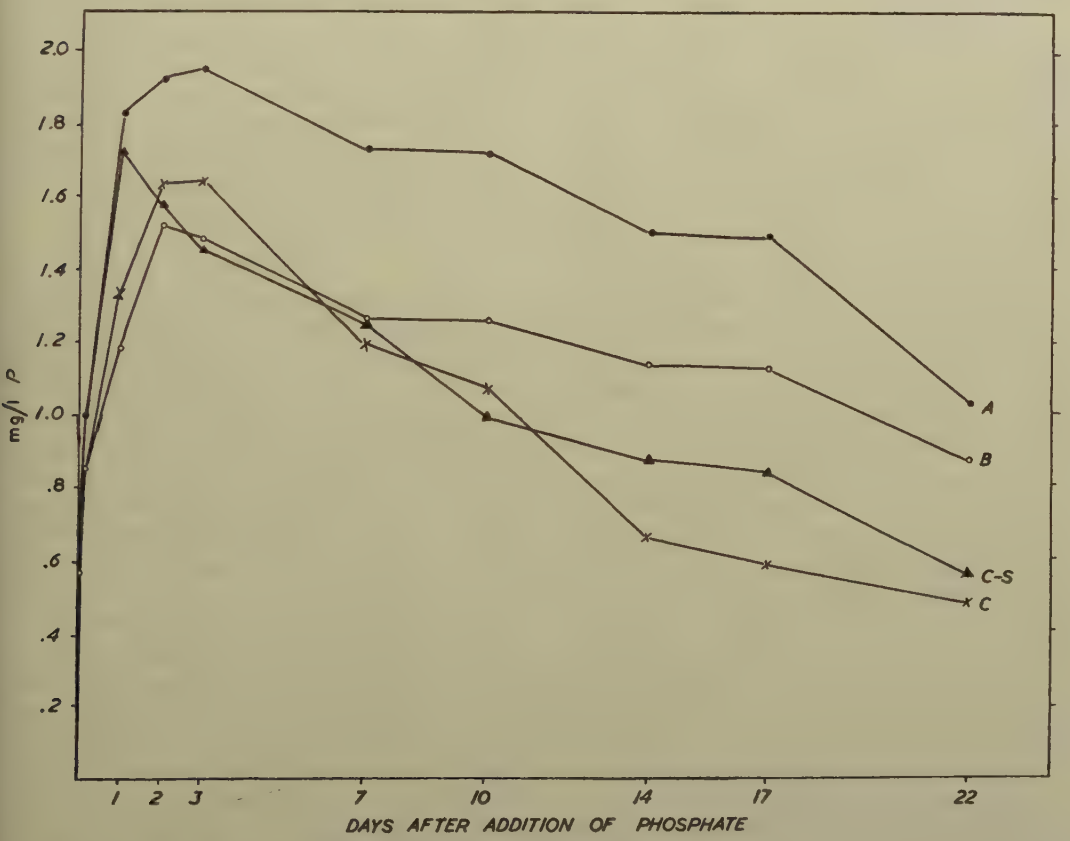

FIG. 2. The effect of different soils on the concentration of phosphorus in water in a laboratory experiment. *A*—control, without any soil; *B*—calcareous soil; *C*—non calcareous clay soil; *C–S*—same as *C* but sterilized.

an equilibrium was reached between the soil and the water, and that further decrease in concentration is due to the same factor as in the control. The treatment containing the soil poor in calcium (*C*) showed a smaller initial fixation of phosphorus than the calcareous soil, but fixation continued during the experiment, resulting in a final phosphorus concentration of 46.8% that of the control.

The difference in phosphate concentration in the sterilized (*C–S*) and the unsterilized (*C*) soils was noticed only after the 10th day of the experiment. At the end of the experiment the concentration with the unsterilized soil was 14% lower than with the sterilized soil. It would seem from this that the effect of soil microorganisms is a delayed one and is very small for the first few days.

The alkalinity and pH showed marked changes in all treatments during the experiment. The bicarbonate alkalinity (to methyl orange) in the control fell from 213 mg/L $CaCO_3$ at the beginning of the experiment to 147.1 mg/L at the end. The carbonate alkalinity (to phenolphthalein) showed an increase from 0 at the beginning to 75.5 mg/L $CaCO_3$ at the end. The pH increased from 8.4 to 8.9. These changes were also noticed in the treatments containing soils but to a lesser degree: the pH increased to 8.8. It seems that these changes are due to the heating of the water containers which were standing outside unshaded. As the temperature of the water rises its capacity to dissolve carbon dioxide decreases, and part of the free carbon dioxide is given off. The equilibrium of the carbonate-bicarbonate system is dependent to a large extent on the carbon dioxide tension in the water. Lowering of this tension causes transfer of bicarbonate to carbonate, part of which may precipitate in the case of calcium carbonate (Pia 1933). Thus the decrease in the CO_2 tension in the water caused a lowering of the methyl orange alkalinity and an increase of the phenolphthalein alkalinity and pH. In the pond the same process is occurring to a greater extent, as CO_2 is also taken up by the phytoplankton during photosynthesis increasing the pH to 9.2–9.5, and cases of a pH as high as 10 or 10.1 have been noted by the author.

As was mentioned before, care was taken in the experiment not to disturb the water in the containers, but in the ponds conditions are different. The action of the wind on the surface of the pond causes a circulation and mixing of the water. The fish by their search for food which is mainly bottom fauna raise up into the water large amounts of mud. Does this have any effect on the equilibrium reached between the phosphorus concentration in the water and the soil? Another problem arising from this first experiment is what are the factors causing the decrease in the phosphorus concentration in the control which contained no soil? A further query is the role of calcium carbonate in the fixation of phosphorus from the water. It must be remembered that although the soils used were different in respect to their calcium carbonate content they also differed in other ways (such as the percentage of finer clay particles), and hence they cannot be compared directly. These points are dealt with in the next experiment which was carried out in a similar way except that the treatments were different.

Experiment 2

In order to find out whether the decline in phosphorus concentration in the control and the other treatments in the last experiment was caused by bacterial action in the water, the water in all the treatments in this experiment was sterilized. The sterilization was done by the addition of 1 liter of 40% formaldehyde to 15 liters of tap water. Sterilization by heat was avoided so as not to change the chemical properties of the water.

The treatments in this experiment were:

(*A*) Control: water without any soil.

(*B*) Soil poor in calcium carbonate [designated as (2) in the soil description]. Special care was taken not to disturb the water and the soil.

(*B–M*) The same soil as (*B*) but the water and soil were mixed well at least once a day.

(*C*) The same soil as (*B*) with the addition of 30% precipitated $CaCO_3$

which was well mixed in with the soil. Again special care was taken not to disturb the water and soil.

(*C–M*) The same as (*C*) mixed well at least once a day.

(*D–M*) Calcareous soil [soil type (1)] mixed well at least once a day.

The results of this experiment are given in Figure 3. From the data given in the figure it can be seen that the mixing of the water and soil caused a much higher and more rapid fixation of the phosphorus by the soil. The treatment containing the soil poor in $CaCO_3$ which was not mixed (*B*) showed scarcely more fixation than the control. The treatment containing the same soil but which was mixed (*B–M*) showed a marked decrease in phosphorus remaining in solution: after 7 days it was 24.4% that of the control and after 14 days 27.4%.

In the unmixed treatments the initial period for the complete solution and dispersion of the phosphorus was seen to be, as in the first experiment, two days. No such period was seen, of course, in the mixed treatments.

The phosphorus concentration in the control, after reaching a peak on the second day, giving a full recovery of the amount added (1.998 mg P/L), was constantly decreasing as in the first experiment. Since this decrease could not have been caused by bacterial activity because the water had

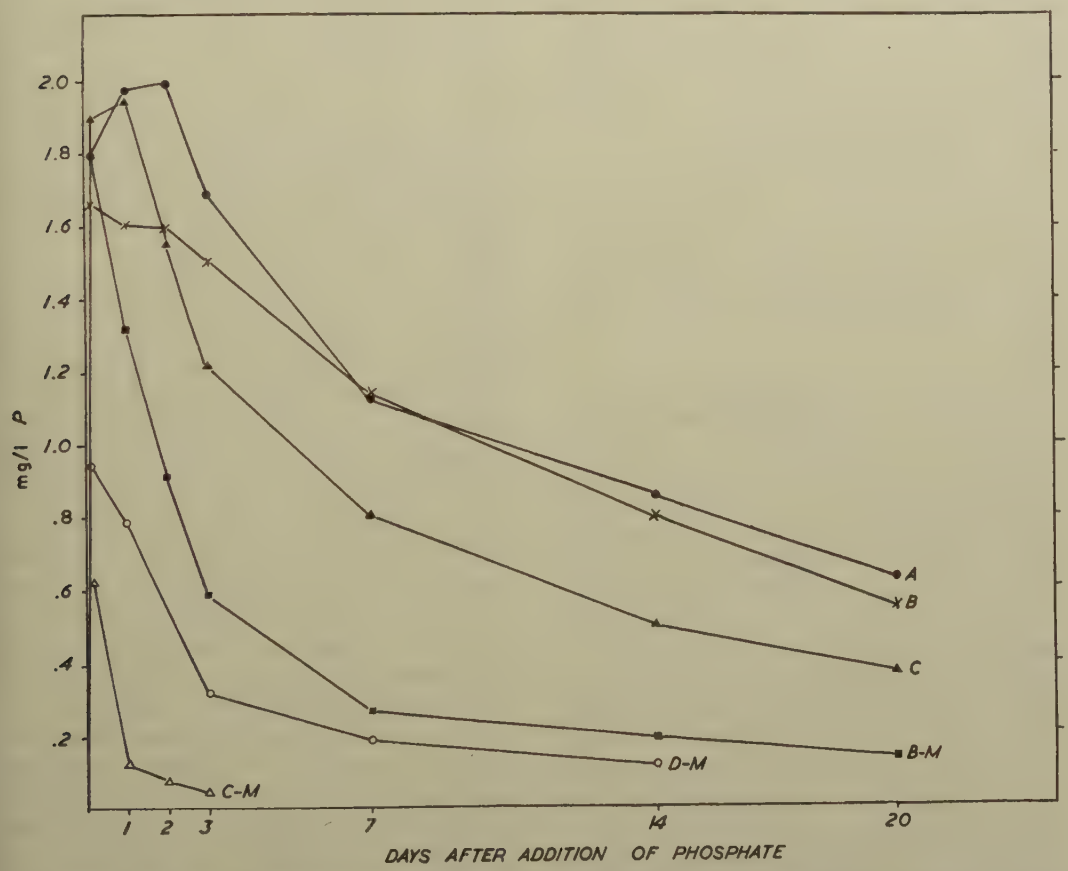

FIG. 3. The effect of added calcium carbonate and the mixing of the soil with the water on the phosphorus concentration in the water in a laboratory experiment. *A*—control, without any soil; *B*—non-calcareous clay soil not mixed with the water; *B–M*—same as *B* but mixed well with the water; *C*—soil same as *B* with 30% $CaCO_3$ added, not mixed; *C–M*—same as *C* mixed well; *D–M*—calcareous soil mixed well.

been sterilized, it would seem to be due to chemical processes.

The $CaCO_3$-poor soil with the addition of 30% $CaCO_3$ and which was not mixed with the water (*C*) showed a very similar effect on the phosphorus concentration as the calcareous soil in Experiment 1—fixation of 22.0% of the phosphorus, compared with the control, by the second day and a subsequent steady decrease roughly parallel with that in the control. Compared with this last treatment the one containing the same soil but which was mixed with the water (*C–M*) showed a very rapid decrease in phosphorus concentration. After only 3 hours 68.8% of the phosphorus added had been fixed by the soil. Twenty-four hours after the addition of the phosphorus this treatment showed a water soluble phosphorus concentration of only 6.4% that of the control, and by the end of the third day it had dropped to 2.8%.

A similar effect although to a lesser extent was observed in the treatment containing calcareous soil (*D–M*).

It can be clearly seen that the intermixing of soil and water increases the fixation of phosphorus by the soil and this is further increased by the presence of $CaCO_3$ in the soil.

The decrease in the phosphate concentration in the water in the control as well as in the treatments containing soil was parallel to changes in alkalinity and pH. These changes followed the same pattern as in the first experiment. The bicarbonate alkalinity (methyl orange alkalinity) decreased in the control from 185.2 mg $CaCO_3$ per liter to 159.1, whereas the carbonate alkalinity (phenolphthalein alkalinity) increased from 23.8 mg $CaCO_3$ per liter to 61.8. This was paralleled by an increase in pH from 8.4 to 9.0. Similar changes in alkalinity and pH were observed in the treatments containing soil but they were not quite so marked: the final pH in these was 8.6–8.8.

The fact that the changes in phosphate concentration and alkalinity occurred in a parallel fashion suggested that they are related in some way. This relationship was studied in the next experiment.

Experiment 3

As was mentioned above, the changes in alkalinity and pH of the water are caused by changes in CO_2 concentration in the water. As the water is heated less carbon dioxide can dissolve in it and part of the carbon dioxide in the water escapes into the air. The uptake of carbon dioxide by the phytoplankton in the fishpond during photosynthesis will further reduce its concentration in the water.

To prevent changes in alkalinity and pH of the water in the experiment we have to stop the escape of carbon dioxide. The effect of preventing the changes in alkalinity and pH on the phosphorus concentration was studied using sealed glass jars filled with tap water to which phosphate was added. These were compared with similar jars kept open to the air. A further series of jars containing distilled water to which the same amount of phosphate was added and which were kept open to the air were also studied. All jars were kept at a constant temperature of 28°C for 8 days and then at 35°C for a further 5 days.

The results of the alkalinity, pH, and phosphorus determinations are given in Table 1. From the data in this table it can be seen that no marked changes in alkalinity and pH occurred in the sealed containers or the distilled water, in the first by preventing the escape of carbon dioxide and in the second by the lack of appreciable amounts of carbonates at all. In these cases no change in the concentration of the phosphorus was noticed, whereas in the open jars containing tap water the usual changes were again apparent: the bicarbonate alkalinity decreased, the carbonate alkalinity increased, and the pH also increased with a consequent decrease in phosphate concentration. These changes were greater when the temperature was raised. This shows clearly the connection between the alkalinity and pH and the phosphorus concentration. As the pH becomes higher the phosphate is removed from solution and is precipitated. The mechanism of this process will be discussed later.

TABLE 1. *Alkalinity (mg $CaCO_3$ per L), pH, and phosphate (mg P/L) in sealed and unsealed jars containining tap and distilled water*

Treatment	Bicarbonate alkalinity			Carbonate alkalinity			pH			Phosphate		
	Initial	After 8 days 28°C	After 5 days 35°C	Initial	After 8 days 28°C	After 5 days 35°C	Initial	After 8 days 28°C	After 5 days 35°C	Initial	After 8 days 28°C	After 5 days 35°C
Tap water—open	175.8	149.6	133.0	0.0	trace	19.	7.65	8.2	8.4	1.66	1.54	1.22
Tap water—sealed	180.5	181.0	173.4	0.0	0.0	0.0	7.6	7.5	7.5	1.66	1.61	1.61
Distilled water—open	trace	trace	trace	0.0	0.0	0.0	6.6	6.6	6.6	1.61	1.62	1.58

Experiment 4

As shown above, the intermixing of soil and water in the laboratory experiments increased the rate of fixation of phosphorus by the soil. This mixing is done in the fishpond by the carp as they search for food in the bottom mud. Schäperclaus (1933) points out that the turbidity of the water can be caused by the food-searching habits of the carp.

It would be interesting to find out the effect of the fish by their mixing action on the phosphorus concentration in the water. Six ponds were used in the study of this problem each being 400 m^2 in area. They were divided into two groups of three, each receiving a different treatment. The ponds for each group were chosen at random. Both groups of ponds were filled with water on the same day. Fourteen days later 120 fish with an average weight of 250 g were put into each pond of the first group but not into the other. The water of all the ponds was found to have about 0.006 mg P/L, with only small deviations in the different ponds. All ponds were then fertilized with 8 kg superphosphate (14.6% P_2O_5) per pond. Further determinations of the phosphates were made in the ponds, and the results are given in Figure 4.

From the data in this figure it can be seen that phosphorus concentration in the ponds containing fish decreased more rapidly than in the ponds without fish. This decrease in phosphorus concentration was less rapid than is usually observed in the ponds, but this is due to the fact that in the short time (2 weeks) since the ponds were filled the phytoplankton population had not developed fully, and hence the pH of the

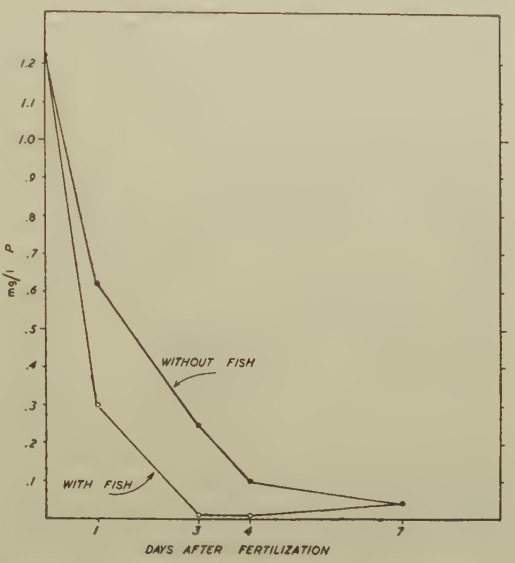

FIG. 4. The effect of mixing by carp of bottom mud with water in fishponds on the phosphorus concentration in the water after fertilization.

water was not so high as is found when the water is more "aged." There was a slight increase in the phosphorus concentration in the pond with fish between the 4th and 7th day to 0.04 mg P/L. The cause of this latter increase is not fully understood. The ponds which contained fish were distinctly more turbid than the ponds without fish.

Experiment 5

The soils used in the foregoing experiments were arable soils, and it was therefore a point of interest to study the properties of pond mud in respect to phosphate fixation. Two soils were introduced into liter jars, which were then filled with tap water. One of these was a bottom mud

taken from a pond at Messiloth in the Beith Shan valley [soil type (3)] and the other was taken from the bank of the same pond [soil type (4)]. From the composition of these soils given on p. 86 it can be seen that both are calcareous soils containing 32.7–32.9% $CaCO_3$. They differ in that the amount of silt and clay particles and organic matter are all higher in the pond mud. The finer particles of the soil are responsible for the adsorptive power of the soil, and hence it was expected that the mud would show a higher fixing capacity.

Phosphate was added to the water in both treatments at a rate of 20 mg superphosphate per liter. This was sufficient to raise the phosphate concentration to 2.28 mg P/L. In order to drive the carbon dioxide more quickly out of the water the temperature of the water in the jars was raised to about 35°C. This caused a rise in the pH as can be seen below:

	before exp.	after 1 day	after 2 days	after 3 days	after 4 days	after 7 days	after 10 days
Bottom mud	7.92	8.41	8.68	8.80	8.81	8.85	8.87
Outside soil	7.90	8.64	8.80	8.84	8.80	8.88	8.87

The results of the phosphate determination are given in Figure 5. From this figure it can be seen that contrary to what was expected, no more phosphorus was adsorbed by the mud than by the soil although the former contained a higher percentage of finer particles. There are three possible explanations of this phenomenon: (a) by the lowering of the pH at the beginning of the experiment due to greater quantities of decomposing organic matter in the bottom mud as compared with the outside soil; (b) by the low phosphate fixation capacity of the organic colloidal particles (Ravikovitch 1934, 1939) found in the bottom mud; (c) by a partial saturation of the adsorptive complex of the bottom mud due to regular phosphate fertilization in the years prior to this experiment.

These three factors all tend to counteract the adsorptive power of the finer particles present in the bottom mud.

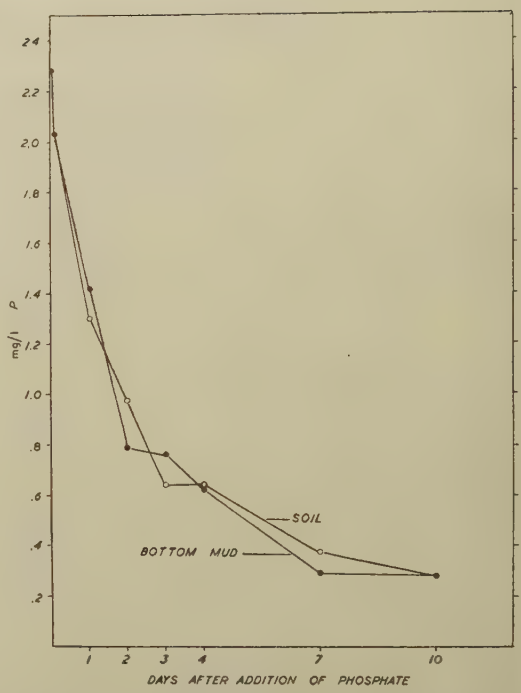

FIG. 5. The effect of fishpond bottom mud and that of adjacent soil on phosphorus concentration in the water in a laboratory experiment.

The phosphate which is removed from solution either directly by the soil or by precipitation should increase the phosphate concentration in the soil. This increase would not be felt in the pond after a single application, as the layer of soil taking part in phosphorus fixation is very thin, according to Hayes *et al.* (1952) not more than 1–2 mm in thickness. The upper layer of the mud is not very distinct, as part of it is suspended in the water; hence when a sample of mud is taken for analysis it is usually a much thicker layer than the "active" layer. The phosphorus concentration added to the mud after fertilization is thus masked in the sample taken.

To overcome this difficulty the mud-water phosphate balance was ascertained in a laboratory experiment in glass jars each containing 5 g of bottom mud [soil type (3)]. The jars were filled with tap water, and then sufficient phosphate was added to raise the phosphorus concentration in the water to 2.28 mg P/L. The concentration of the

TABLE 2. *Phosphate balance in water-mud system in glass jars containing 1 liter of water and 5 g of pond mud*

Water		Mud	
Before the experiment	0.105 mg P/L	Before the experiment	0.667 ppm P
Amount added	2.280 mg P/L	After the experiment	1.000 ppm P
Total	2.385 mg P/L	Amount gained	0.333 ppm P
After the experiment	0.718 mg P/L		
Amount lost	1.667 mg P/L	Amount gained by 5 g mud in one jar	1.665 mg P/L

phosphate in the water as well as in the mud was determined before and after the experiment. From these data the phosphate balance of the mud-water system was calculated (Table 2). As can be seen the amount of phosphorus lost from the water was gained quantitatively by the mud.

DISCUSSION

Although the rapid decline in phosphate concentration in the water of fishponds has been noted by many workers (Breest 1921, Demoll 1925, Nisbet 1951, Zeller 1952), not many studies on the causes of this decline have been made in fishponds, and usually assumptions were made from the results of studies made in lakes. Ponds and lakes, although both freshwater habitats, differ greatly with respect to their physical and chemical natures. Physical properties such as depth, ratio of volume of water to area of mud surface, and thermal stratification all have a considerable effect on the phosphorus cycle. The results of studies on the phosphorus cycle in lakes should not therefore be applied to ponds without a critical examination. Nevertheless they give a very important line of approach to the corresponding problems in the ponds.

What are the main causes of a fall in phosphorus concentration in a lake? Firstly the uptake of phosphorus by the phytoplankton. This is considered by some authors (Einsele 1941, Smith 1945, Coffin *et al.* 1949, Hutchinson and Bowen 1950) to be the main cause for the decline in phosphate concentration after the fertilization of the epilimnion of the lake. Coffin *et al.* (1949) point out that this uptake of phosphorus by the phytoplankton takes place in the short space of a few hours or even minutes. In most cases, however, the decrease in concentration of phosphorus in the water after fertilization was not associated with a corresponding increase in the phytoplankton population as ought to be the case if the phosphorus was used by the phytoplankton. When an increase in phytoplankton was seen it did not take place until some months later, two months after fertilization in Smith's experiment and after three months in Einsele's experiment. Einsele (1941) suggests that phosphorus is absorbed by the phytoplankton in an "active" physiological way. This phosphorus does not become part of the organic matter building up the cell but is stored in the cell for further use. In this way, Einsele points out, the phytoplankton cells can store in 24 hours ten times as much phosphorus as is usually found in the water. Mackereth (1952) expressed a similar view on the phosphorus storing ability of *Asterionella formosa*, as did Goldberg *et al.* (1951) working with *Asterionella japonica*. However, Mackereth suggests that this uptake of phosphorus may be a purely physical phenomenon by adsorption on some part of the cell tissue. This idea is supported by the fact that Goldgerg *et al.* found that a considerable amount of the cell phosphorus is in a labile state and can be washed out with water.

This ability of the phytoplankton to absorb the phosphate from the water very rapidly and store it for further use is of prime importance in the consideration of the fate of the phosphate added to fishponds. Although the "inorganic" fixation of phos-

phorus in the water and soil is rapid, there is a short period after fertilization when the phosphorus is still in solution and when the phytoplankton can absorb a considerable amount of it. If the amounts of phosphate normally added to the fishponds in Israel in a single fertilization (approx. 0.5 mg P/L) are compared to the phosphate concentrations cited in the papers mentioned above, it will be seen that they are ten times as much as the maximum concentration given by Mackereth and 50–80 times the concentration usually found in the water before fertilization. The high concentration of phosphorus disappears in 24 hours and in many cases in a much shorter period. Ketchum (1939) found a linear relationship between phosphorus concentration in the cells of *Nitzschia closterium* and phosphate in the medium, but at concentrations above ca. 0.1 mg PO_4/L (0.033 mg P/L) in the medium no further increase in phosphate concentration in the cell was noted (cf. Ketchum 1939, Fig. 2). It seems from the above discussion that even if a rapid "active" phosphorus uptake does take place in the pond its effect on the disappearance of the high concentration of phosphorus after fertilization is small, and the main effect can be attributed to other causes such as adsorption by the bottom mud and chemical processes in the water resulting in phosphate precipitation.

Studies on the phosphate cycle in the hypolimnion of lakes showed the importance of the role of the bottom mud (Mortimer 1941, 1942, 1949; Einsele 1938, 1941; Hayes *et al.* 1952). The mud adsorbs or releases the phosphate according to the conditions in the hypolimnion. According to Hayes *et al.* (1952) the turnover time of phosphorus is less in small and shallow lakes. The importance of the mud in adsorbing phosphate in fishponds was stressed by Breest (1921), Lantzsch (1924), and Demoll (1925). The results of the present study emphasize the importance of the mud in the phosphorus cycle of ponds.

Most of the papers mentioned above consider the mechanism of this adsorption to be connected with oxidized ferric compounds in the mud such as ferric hydroxide, ferric phosphate, and ferric-organo-complex compounds. Mortimer writes (1941: 324): "The strongly adsorbent properties of lacustrine muds, especially in the surface layers, have long been recognized in commercial fishpond practice. . . From the experiment described here and also from observation during the winter of 1939 in Esthwaite Water there is reason to suppose that this adsorbing influence is exercised by the oxidized mud surface." Phosphate adsorption to these ferric compounds, however, takes place only under certain conditions of pH. Ohle (1937) showed the ampholytic properties of ferric hydroxide gel which is electro-positive in acid pH and electro-negative in alkaline pH. The adsorption of negatively charged phosphate ions onto the ferric hydroxide can happen therefore only in the acid range of the pH. In his experiments 65% of the phosphate added to ferric hydroxide gel (suspension) was retained and adsorbed to the ferric compound at pH 6 but none at all at pH 9.

The results of Ohle's experiments are in agreement with results of studies made on phosphate adsorption by ferric compounds in arable soils. Perkins (1947) found a decrease in phosphate fixation by iron as the pH increases. Bradfield *et al.* (1935) quoted by Dean (1949) points out that maximum phosphate fixation by iron and aluminum takes place at pH 2–5 and declines when the pH is higher. Ohle (1938) stressed the importance of manganic compounds in phosphate adsorption but again no adsorption was found by him at pH 8. Perkins (1947) found maximum fixation by magnesium to be at pH 4.0, decreasing as the pH increased.

The low pH values found almost always in the hypolimnion of lakes is caused by the decomposition of organic matter with the subsequent release of CO_2 and organic acids. This low pH is also found very often in fishponds in temperate regions but never in subtropical Israel, where ponds are usually situated on calcareous soils. The minimum pH in these ponds is normally 7.5–8.0, and on summer days the pH may reach 9.0–9.5 (Shilo and Shilo 1953). On some occasions values as high as 10.1

have been recorded by the author in experimental fishponds.

It is clear that phosphate adsorption on compounds of Fe, Mn, and Al although possible in fishponds where the pH is low, *e.g.* in Europe and the Far East, is impossible in the high pH of the fishponds in Israel. Moreover, because of the high pH and oxygen concentration in the water the amounts of these metals in the water or in the upper layers of the mud is very small. In these circumstances a different explanation must exist for the mechanism of the phosphorus fixation by the mud as shown by the results of the present study. Such an explanation is found in the results of studies on phosphorus fixation by arable soils. Physico-chemical adsorption onto soil colloids as an anion-exchange reaction has been described by Ravikovitch (1934), Dean and Rubins (1947), Paul (1949), and Dean (1949). In calcareous soils the added phosphate also reacts with the calcium carbonate to form insoluble calcium phosphate compounds (Brackett and Freeman 1915, Magruder 1917, McGeorge and Breaseale 1932, Buehrer 1932, McGeorge *et al.* (1935), McGeorge 1939, MacIntire 1941, and Lewis *et al.* 1950).

The adsorptive capacity of the soil or mud is determined by its composition as well as by the conditions, such as pH, phosphorus concentration added, degree of saturation, etc., prevailing in it. It can be seen from the results of the foregoing experiments that although phosphorus is adsorbed by the soil with a very low $CaCO_3$ content, this adsorption is much higher when $CaCO_3$ is present. It seems reasonable to expect that the higher the colloid and $CaCO_3$ contents of the mud the higher the phosphorus adsorption will be in the same conditions. However, the nature of these colloids has a large effect on the adsorptive capacity of the mud. Ravikovitch (1934, 1939) showed that organic colloids have a very low adsorptive capacity compared with mineral clay colloids. The organic colloids also have an indirect effect on phosphorus adsorption. Their decomposition regenerates phosphorus which counteracts their adsorptive role, and carbon dioxide is also evolved which lowers the pH in the mud hence reducing the fixation of phosphorus by the $CaCO_3$.

As a fishpond gets older more organic matter is deposited on the bottom mud. The amount of colloids is increased but its nature is changed in such a way that it does not effect the adsorptive capacity of the mud to any great extent, as was shown in the experiments in the present study. Another point which should also be considered is that by constant fertilization of a pond the colloids become partially saturated. As a result of these processes an old pond can even show a decrease in the adsorptive capacity of the mud. Lantzsch (1924) gives some figures for the adsorptive capacity of different soils to methyl violet in proportional values of 1.6 for sandy soils, 49 for arable soil and 241 for pond bottom soil. Demoll (1925) postulates from these results on the ability of pond bottom mud to adsorb phosphate. The disagreement between Lantzsch's results and those found in this paper may be explained by different properties of the adsorbing agent and especially the different compounds being adsorbed.

One interesting point in the results of the experiments is the effect of mixing the mud with the water on the phosphate adsorption by the mud. By this mixing, water containing phosphate comes into contact with more mud particles, and the transfer of the phosphorus from the water to the mud is more rapid and complete. This mixing affects the fixation of phosphorus by $CaCO_3$ more than by the anion exchange process as can be seen from the data in Table 3.

In the experiments described there was a constant decrease in phosphate concentra-

TABLE 3. *Phosphorus concentrations in the water after mixing with soil expressed as percentage of the concentration in an unmixed system containing the same soil*

Soil	Soil type	Days after addition of phosphate				
		1	2	3	7	19
Soil poor in Ca	2	82.7	57.0	39.4	23.6	24.1
Soil poor in Ca + 30% $CaCO_3$	2	6.5	5.5	3.8	—	—
Calcareous soil	1	61.5	25.0	22.6	17.6	—

tion in the water in the control which contained no soil. This proved to be caused by precipitation of phosphate due to the CO_2 tension in the water and changes in pH. Gessner (1939) described such a precipitation in the water of the River Danube, and he assumed the precipitate to be $Ca_3(PO_4)_2$. This precipitation takes place as the pH is raised by the uptake of CO_2 by the phytoplankton during photosynthesis. Armstrong and Harvey (1950) mention a similar process in the English Channel, and they also quote Dietz *et al.* (1942) who found such a process taking place in the Pacific Ocean. Barrett (1953) found a correlation between the alkalinity of a lake and the phosphate concentration after the addition of phosphate, thus confirming Naumann's (1932) classification of alkalitrophic lakes.

Since the anion PO_4^{---}, which according to Gessner is precipitated as $Ca_3(PO_4)_2$, is present only in very small quantities it would be of interest to examine this assumption from a theoretical standpoint. Buehrer (1932) discussed the relations of various phosphate ions in a similar way to that described below.

Phosphate in solution undergoes dissociation in three stages:

$$H_3PO_4 = H^+ + H_2PO_4^- \quad (1)$$

$$H_2PO_4^- = H^+ + HPO_4^{--} \quad (2)$$

$$HPO_4^{--} = H^+ + PO_4^{---} \quad (3)$$

These three anions are in an equilibrium which is determined by their dissociation constants according to the following equations:

$$\frac{[H^+] \times [H_2PO_4^-]}{[H_3PO_4]} = K_1 \quad (4)$$

$$\frac{[H^+] \times [HPO_4^{--}]}{[H_2PO_4^-]} = K_2 \quad (5)$$

$$\frac{[H^+] \times [PO_4^{---}]}{[HPO_4^{--}]} = K_3 \quad (6)$$

The concentrations of the different ions are in mols.

It is clear that the concentration of H^+ in the water is expressed by the pH of the water and is the same in all three equations. The amounts and ratios of the three phosphate anions will change with variations in the pH of the water but will remain constant at a given pH. These amounts can be calculated from the corresponding values of K_1, K_2, and K_3.

Differences between the values given in different papers are not very great. The ionic dissociation constants $K_1 = 1.1 \times 10^{-2}$, $K_2 = 2.0 \times 10^{-7}$, and $K_3 = 3.6 \times 10^{-13}$ (Abbot and Bray 1909, Treadwell and Hall 1945, Tananaev 1950) were taken as a basis for the calculation of the amounts of the three different phosphate ions in solution. Table 4 gives the amounts of these ions in solution containing 1 mg P/L at various levels of pH. The amount of PO_4 ion although small at low pH becomes greater with increasing pH.

The amount of the PO_4 ion which can go into solution as the soluble part of $Ca_3(PO_4)_2$ is defined by the solubility product according to the equation:

$$[Ca^{++}]^3 \times [PO_4^{---}]^2 = S_{Ca_3(PO_4)_2} \quad (7)$$

where $S_{Ca_3(PO_4)_2}$ is the solubility product, and the amounts of ions in solution are expressed in molals. The amount of the ion PO_4 which is soluble in water is dependent on the amount of Ca ion in the solution, and this can be calculated from the following equation derived from the previous one:

$$[PO_4^{---}] = \sqrt{\frac{S_{Ca_3(PO_4)_2}}{[Ca^{++}]^3}} \quad (8)$$

Britton (1927) found the value of $S_{Ca_3(PO_4)_2}$ to be 1×10^{-25}, Zharovskii (1951) gives 2.0×10^{-29}, and Latimer (1952) who based his calculation on thermodynamic data gives 1.3×10^{-32}. Calculations on the solubility of phosphorus ions in this paper

TABLE 4. *Amounts of phosphate ions (mg P/L) in a solution containing 1 mg phosphate P/L* (For method of calculation see text.)

pH	H_3PO_4	$H_2PO_4^-$	$HPO_4^=$	$PO_4^{\equiv}$
7.0	0.303×10^{-5}	0.333	0.666	0.240×10^{-5}
8.0	0.433×10^{-7}	0.476×10^{-1}	0.952	0.343×10^{-4}
9.0	0.452×10^{-9}	0.497×10^{-2}	0.994	0.358×10^{-3}
10.0	0.452×10^{-11}	0.498×10^{-3}	0.996	0.358×10^{-2}

are based on Britton's figure, but it should be borne in mind that the lower figures may be more correct.

Table 5 shows the amounts of PO_4 ions in solution at different concentrations of Ca ions. As long as the pH of the water is low the amount of PO_4 ions formed by the dissociation of the phosphate is low and usually does not exceed the amount which can dissolve; but as the pH of the water rises to higher values the amounts of PO_4 ions tend to be larger than can be dissolved, especially when the amount of Ca ion in the water is high, and hence part of the PO_4 ions is precipitated as $Ca_3(PO_4)_2$. For example, in water containing 50 mg Ca^{++}/L and at pH 8.0, 0.343×10^{-4} mg P/L of PO_4 ion is formed (Table 4). In this water 0.222×10^{-3} mg P/L of PO_4 ion can go into solution (Table 5), so that all the PO_4 ions formed will stay in solution. If the pH rises to 9.0, 0.358×10^{-3} mg P/L of PO_4 ion are formed. This amount exceeds the quantity that can go into solution, and part of it, in this case 0.136×10^{-3} mg P/L, is precipitated. This part would be larger in a higher concentration of Ca^{++}.

As was mentioned before, the ratio of the three phosphate ions is constant at a given pH, and the precipitation of part of the PO_4 ions will upset this equilibrium. Equilibrium is restored by the formation of new PO_4 ions by dissociation from the first two ions, but this will be precipitated until the amount of PO_4 ions in the saturated solution is in the right proportion with the other two ions. Thus not only the amount of PO_4 ions is affected by the precipitation but also the general phosphate concentration in the water. The maximum concentration of phosphate that can be dissolved in water at different values of pH and Ca ion concentrations can be calculated from the equation:

$$C = \frac{100 \times S_{PO_4}}{A_{PO_4}} \qquad (9)$$

where C = the total phosphate concentration in the water, S_{PO_4} = the PO_4 ion concentration in its saturated solution at given Ca ion concentration, and A_{PO_4} = the percentage of PO_4 ion concentration in the total phosphate concentration for a given pH. The total phosphate concentration soluble in water as calculated for different pH values and Ca ion concentrations are given in Figure 6.

A comparison between phosphate concentrations found in treatments containing no soil and those derived by calculation for the same conditions is given in Table 6. The agreement between the two sets of figures is fairly good, taking into account the fact that Ca ion concentration values were taken from the general analysis of the water and not from determinations during the experiments.

In fishponds there is a diurnal fluctuation of pH of the water brought about by the

TABLE 5. *Concentration of phosphate in saturated solution of $Ca_3(PO_4)_2$ at different concentrations of Ca ions in the solution ($S_{Ca_3(PO_4)_2} = 10^{-25}$)*

Ca^{++} (mg/L)	$PO_4^{\equiv}$ (mg P/L)
50	0.222×10^{-3}
100	0.786×10^{-4}
150	0.419×10^{-4}
200	0.278×10^{-4}
250	0.199×10^{-4}
300	0.151×10^{-4}

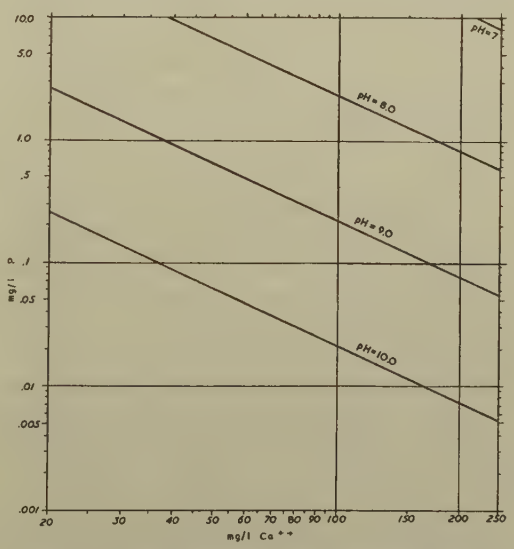

FIG. 6. Total soluble phosphorus concentrations calculated for water of different pH values and calcium ion concentrations. For method of calculation see text.

TABLE 6. *Phosphorus concentration in treatments containing no soil compared with figures derived by calculation*

Exp. no.	pH	Ca^{++} mg/L	P concentration found, mg/L	P concentration calculated, mg/L
1	8.9	52	1.029	0.712
2	9.0	52	0.624	0.586
3	8.4	98	1.217	1.148
A*	8.6	98	0.234	0.587
B*	9.0	98	0.163	0.228

* From another series of experiments not mentioned in this paper.

photosynthetic activity of the phytoplankton and respiration of total biota. Cerny (1948) found the pH in a small European fishpond to fluctuate between 6.7 and 8.5. As can be seen from Figure 6 the amount of phosphorus that can be dissolved without being precipitated as $Ca_3(PO_4)_2$ is high, considering the low concentrations of Ca ion usually found in these waters (although phosphate might be precipitated or adsorbed by other means mentioned in the earlier parts of this discussion). In Israel, however, the pH usually reaches a much higher value, as mentioned previously, values of 9.2–9.4 (Shilo and Shilo 1953) and even higher being not uncommon. With these high pH values and the high calcium content of the water precipitation of phosphate must be very large and is probably the main cause of the disappearance of phosphate after fertilization.

CONCLUSION

Fishponds in Israel differ from fishponds in the more humid areas and temperate zones by having more alkaline water, especially during summer days, and a high content of calcium both in water and in the bottom mud. Under these conditions phosphate added to the water in soluble form disappears rapidly from the water mainly by being precipitated as calcium phosphate. This process is influenced by the pH and the calcium content of the water.

A certain amount of the added phosphate is adsorbed by the bottom mud and soil. This is thought to be brought about mainly by an anion exchange process with the colloidal matter of the mud and by chemical reaction with calcium carbonate in the soil and mud. Adsorption onto heavy metals (as found in ponds in temperate regions) is less likely to occur because of the high pH. Mixing of the bottom mud and water increases phosphate fixation by the mud especially by the calcium carbonate.

As organic colloids have a much smaller phosphate-adsorption capacity than clay colloids, the accumulation of organic colloids on the bottom in old ponds does not increase the adsorptive capacity of the mud although the amount of colloidal matter in the mud is increased. It is to be expected that in older fishponds the adsorption of phosphate by the mud will even decrease as a result of accumulation of organic matter on the bottom and partial saturation of the adsorption complex by regular fertilization with phosphate.

REFERENCES

ABBOT, G. A., AND W. C. BRAY. 1909. The ionization relations of orth- and pyrophosphoric acids and their sodium salts. J. Amer. Chem. Soc., **31**: 729–763.

AMERICAN PUBLIC HEALTH ASSOCIATION. 1946. Standard methods for the examination of water and sewage. New York, xvii + 286 pp. (9th ed.)

ARMSTRONG, F. A. J., AND H. W. HARVEY. 1950. The cycle of phosphorus in the English Channel. J. Mar. Biol. Ass. U. K., **29**: 145–162.

BARRETT, P. H. 1953. Relationships between alkalinity and adsorption and regeneration of added phosphorus in fertilized trout lakes. Trans. Amer. Fish. Soc., **82**: 78–90.

BRACKETT, R. N., AND B. FREEMAN. 1915. A note on the formation of tricalcium phosphate on mixing ground limestone with acid phosphate. J. Indust. Eng. Chem., **7**(7): 620.

BRADFIELD, R., G. D. SCARSETH, AND J. G. STEELE. 1935. Factors affecting the retention of phosphate by clays. Trans. 3rd Intern. Congr. Soil Sci., **1**: 74–75.

BREEST, F. 1921. Studien über die Phosphorsäuregehalt im Boden und im Wasser. I. Über die Nachwirkung von Phosphorsäuredüngung und gelöste Phosphorsäure im Teich. Int. Mitt. Bodenk., **11**: 111–116.

BRITTON, H. T. S. 1927. Electrometric study of precipitation of phosphate. J. Chem. Soc., **1927**: 614–630.

BUEHRER, T. F. 1932. The physico-chemical relationships of soil phosphates. Tech. Bull. Ariz. Agric. Exp. Sta., **42**: 155–212.

CERNY, A. 1948. Zur Dynamik von Seichtgewässern. Schweiz. Z. Hydrol., **10**(4): 36–52.

COFFIN, C. C., F. R. HAYES, L. H. JODREY, AND

S. G. Whiteway. 1949. Exchange of materials in lakes as studied by the addition of radioactive phosphorus. Canad. J. Res., Sect. D., **27**(4): 207–222.

Dean, L. A. 1949. Fixation of soil phosphorus. Advanc. Agron., **1**: 391–411.

Dean, L. A., and E. J. Rubins. 1947. Anion exchange in soils. I. Exchangeable phosphorus and anion exchange capacity. Soil Sci., **63**(5): 377–387.

Demoll, R. 1925. Teichdüngung. Handb. Binnenfisch. Mitteleur. A., **5**: 53–160.

Dietz, R., K. Emery, and F. Shepard. 1942. Phosphorus deposits on the sea floor of southern California. Geol. Soc. Amer. Bull., **53**: 815–848.

Einsele, W. 1938. Über Chemische und Kolloidchemische Vorgänge im Eisen-phosphatsystemen unter limnochemischen und limnogeologischen Gesichtspunkten. Arch. Hydrobiol., **33**: 361–387.

———. 1941. Die umsetzung von zugefürtem anorganischer Phosphat in eutrophen See und ihre Rückwirkung auf seinen Gesamthaushalt. Z. Fisch., **39**(3): 407–488.

Fischer, A., and S. Rashkes. 1951. Experiments on the growth of carp in fertilized and unfertilized ponds. Bamidgeh, **3**(45/46): 80–104. (In Hebrew with Engl. summ.)

Gessner, F. 1939. Die Phosphorarmut der Gewässer und ihre Beziehung zum Kalkgehalt. Int. Rev. Hydrobiol., **38**: 203–211.

Goldberg, E. G., T. J. Walker, and A. Whisenand. 1951. Phosphate utilization by diatoms. Biol. Bull. (Wood's Hole), **101**(3): 274–284.

Hayes, F. R., J. A. McCarter, M. L. Cameron, and D. A. Livingstone. 1952. On the kinetics of phosphorus exchange in lakes. J. Ecol., **40**(1): 202–216.

Hutchinson, G. E., and V. T. Bowen. 1950. Limnological studies in Connecticut. IX. A quantitative radiochemical study of the phosphorus cycle in Linsley Pond. Ecology, **31**(2): 194–203.

Ketchum, B. H. 1939. The absorption of phosphate and nitrate by illuminated cultures of *Nitzschia closterium*. Amer. J. Bot., **26**: 399–407.

Lantzsch, K. 1924. Der Boden der Wielenbacher Teiche (Kolloidgehalt, Bakterientätigkeit). Arch. Hydrobiol., **15**: 406.

Latimer, W. M. 1952. The oxidation states of the elements and their potentials in aqueous solution. Prentice-Hall, N. Y., xvi + 392 pp. (2nd ed.)

Lewis, G. L., G. O. Baker, and R. S. Snyder. 1950. Phosphate fixation in calcareous soils. Soil Sci., **69**(1): 55–62.

MacIntire, W. H. 1941. A new explanation of what happens to superphosphate in limed soils. Bull. Tenn. Agri. Exp. Sta., **176**: 16 pp.

Mackereth, F. J. 1952. Phosphorus utilization by *Asterionella formosa* Hass. J. Exp. Bot., **4**(12): 296–313.

Magruder, E. W. 1917. The action of calcium carbonate on acid phosphate. J. Indust. Eng. Chem., **9**(2): 155–156.

McGeorge, W. T. 1939. Factors influencing the availability of native soil phosphorus and phosphate fertilizers in Arizona soils. Tech. Bull. Ariz. Agric. Exp. Sta., **82**: 295–331.

McGeorge, W. T., and J. F. Breaseale. 1932. Studies on iron, aluminum, and organic phosphates and phosphate fixation in calcareous soils. Tech. Bull. Ariz. Agric. Exp. Sta., **40**: 59–109.

McGeorge, W. T., T. F. Buehrer, and J. F. Breaseale. 1935. Phosphate availability in calcareous soils: A function of carbon dioxide and pH. J. Amer. Soc. Agron., **27**(5): 330–335.

Mortimer, C. H. 1941–1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol., **29**: 280–329; **30**: 147–201.

———. 1949. Seasonal changes in chemical conditions near the mud surface in two lakes of The English Lake District. Verh. int. Ver. Limnol., **10**: 353–356.

Mortimer, C. H., and C. F. Hickling. 1954. Fertilizers in Fishponds. Fish. Publ. Lond. **5**: 155 pp.

Naumann, E. 1932. Grundzüge der regionalen Limnologie. Die Binnengewässer, **11**: 176 pp.

Neess, J. C. 1949. Development and status of pond fertilization in Central Europe. Trans. Amer. Fish. Soc., **76**: (1946): 335–358.

Nisbet, M. 1951. Sur le métabolisme des sels minéraux introduits dans les étangs. Verh. Int. Ver. Limnol., **11**: 268–275.

Ohle, W. 1937. Kolloidgele als Nahrstoffregulatoren der Gewässer. Naturwissenschaften, **25**: 471–474.

———. 1938. Die Bedeutung der Austauschvorgänge zwischen Schlamm und Wasser für den Stoffkreislauf der Gewässer. Vom Wasser, **13**: 87–97.

Paul, H. 1949. Some observations on the mechanism of phosphorus fixation in some soils of British Guiana. J. Agric. Sci., **39**(3): 249–253.

Perkins, A. T. 1947. Phosphate solubility in relation to cation and pH: Magnesium. Soil Sci. Soc. Amer. Proc., **12**: 185–187.

Pia, J. 1933. Kohlensäure und Kalk. Die Binnengewässer, **13**: vii + 183 pp.

Rashkes, S. 1949. On the nitrogen and phosphorus in the Asi River and the experimental ponds at Nir David. Alon Lemegadley Dagim, **26/27**: 1–12. (In Hebrew)

Ravikovitch, S. 1934. Anion exchange: I. Adsorption of the phosphoric acid by soils. Soil Sci., **38**: 219–239.

———. 1939. Influence of exchangeable cations on the availability of phosphate in soils. Soil Sci., **47**: 357–366.

Reich, K. 1950. On a small experiment for choosing a fertilization method. Alon Lemegadley Dagim, **32**: 1–9. (In Hebrew)

Rochford, D. J. 1951. Studies in Australian estuarine hydrology. Aust. J. Mar. Freshwater Res., **2**(1): 1–116.

Schäperclaus, W. 1933. Text-book of pond culture. P. Parey, Berlin. Engl. Transl. U. S. Fish & Wildl. Serv. Leafl., **311**: 260 pp.

Shilo, M., and M. Shilo. 1953. Conditions which determine the efficiency of ammonium sulfate in the control of *Prymnesium parvum* in laboratory cultures and in fishponds. Bamidgeh, **5**(1/2): 1–10. (In Hebrew)

Smith, M. W. 1945. Preliminary observations upon the fertilization of Crecy Lake, New-Brunswick. Trans. Amer. Fish. Soc., **75**: 165–174.

Tananaev, N. A. 1950. Drobny analisis. Moskva, 278 pp. (In Russian)

Treadwell, F. P., and W. T. Hall. 1945. Analytical chemistry. II. Quantitative analysis. Wiley, New York.

Wirshubsky, A. 1952. A device for water sampling in ponds. Bamidgeh, **4**(3/4): 54. (In Hebrew).

Zeller, H. D. 1952. Nitrogen and phosphorus concentrations in fertilized and unfertilized farm ponds in central Missouri. Trans. Amer. Fish. Soc., **82**: 281–288.

Zharovskii, F. G. 1951. The solubility of phosphates. Trud. Kom. Anal. Khim. Akad. Nauk S. S. S. R., **3**: 101–115.

Coliform Bacteria in Sediments Around Three Marine Sewage Outfalls[1]

Sydney C. Rittenberg, Tod Mittwer, and Daniel Ivler

Department of Bacteriology and the Hancock Foundation, University of Southern California, Los Angeles 7, California

ABSTRACT

The distribution of coliforms in the sediments around three marine sewage outfalls was investigated. It was found that coliform fields extended for considerable distances around the two outfalls discharging unchlorinated primary treatment effluent and that a less extensive field existed around the outfall discharging more highly treated sewage. In general, the coliforms in the sediments were found in areas beneath the path of movement of the effluent field in the surface water. It is probable that the bulk of the coliforms reach the sediments by sedimentation from the overlying waters. Although the coliforms in the sediments must persist for a sufficiently long period to permit the accumulation of large numbers by deposition from low count waters, no precise estimate of the survival time in the sediment could be made.

INTRODUCTION

It has been the common practice of communities situated on coastline to utilize the adjacent oceans as a convenient receiver for all domestic wastes. At the present time, for example, 37 sewage outfalls in the State of California discharge into the Pacific Ocean (Pearson 1955), the largest of these contributing approximately 250 million gallons of domestic sewage per day to the coastal waters. When public health investigations in the late 1800's made it clear that enteric infections are primarily waterborne, attention was directed to the possible public health significance of the practice of discharging human wastes into marine waters used for recreational purposes or for the culture of shellfish. Numerous investigations were made of the occurrence and survival of enteric pathogens and coliform bacteria in sea water (see Zobell 1941, Pearson 1955, Greenberg 1956, Terry 1956, for extensive reviews) and many public health agencies have established coliform standards for marine waters under their jurisdiction.

However, except for a single report by Nusbaum and Garver (1955) who briefly mention finding up to 24 million coliforms per gram of wet mud in the vicinity of an outfall discharging into San Diego Bay, we have found no published data on the occurrence and survival of coliform bacteria in the sediments adjacent to marine sewage outfalls. During the course of an oceanographic investigation of Santa Monica Bay, conducted to assist in the design of a new ocean outfall for the city of Los Angeles, studies were made on the occurrence of coliforms in the marine sediments around three existing outfalls.

Most of the work reported here was done under contract with the Hyperion Engineers, Los Angeles, but part was supported by a grant from the Los Angeles County Sanitation Districts. Many individuals assisted us in the field work or by providing unpublished information. It is a pleasure to acknowledge the aid of Mr. C. G. Gunnerson, Los Angeles Bureau of Sanitation; Mr. W. Garber of the Los Angeles City Sanitation Laboratories; Mr. L. Nelson and Mr. W. Henderson, Orange County Sanitation Districts; Mr. A. M. Rahn and Mr. F. Bowerman, Los Angeles County Sanitation Districts; Dr. R. E. Stevenson, Dr. R. J. Martinez, Mr. L. I. Hochstein, and Mr. B. Mechalas, University of Southern California; and Captain G. Allan Hancock and the members of the crew of the Velero IV.

[1] Allan Hancock Foundation, contribution No. 204.

AREAS OF STUDY

Sediments in the vicinity of the Orange County, the Los Angeles County, and the Los Angeles City outfalls were investigated. The Orange County Sanitation District outfall is located just upcoast of the mouth of the Santa Ana River (Fig. 1) on an exposed flat coastal plain bordered by sandy beaches. It extends about 7000 feet to sea and discharges approximately 30 million gallons per day of unchlorinated, primary treatment effluent in a depth of 50 feet of water. The ocean bottom in this area slopes gently from shore, and the 50-fathom contour runs about 5 miles off-shore. Sediments in the area consist of medium to fine sands and silty sands. In general the sediments are low in organic matter and appear clean. However, in a small area around the outfall the sediments are rich in organic matter and have a black color due to the presence of ferrous sulfide.

The Los Angeles City (Hyperion) outfall is located between Manhattan Beach and Ballona Creek in the south central section of Santa Monica Bay (Fig. 2). It extends one mile to sea and discharges about 250 million gallons per day of chlorinated secondary treatment effluent in a depth of 50 feet of water. The degree of chlorination is such that a small viable population of coliforms is probably entering the bay most

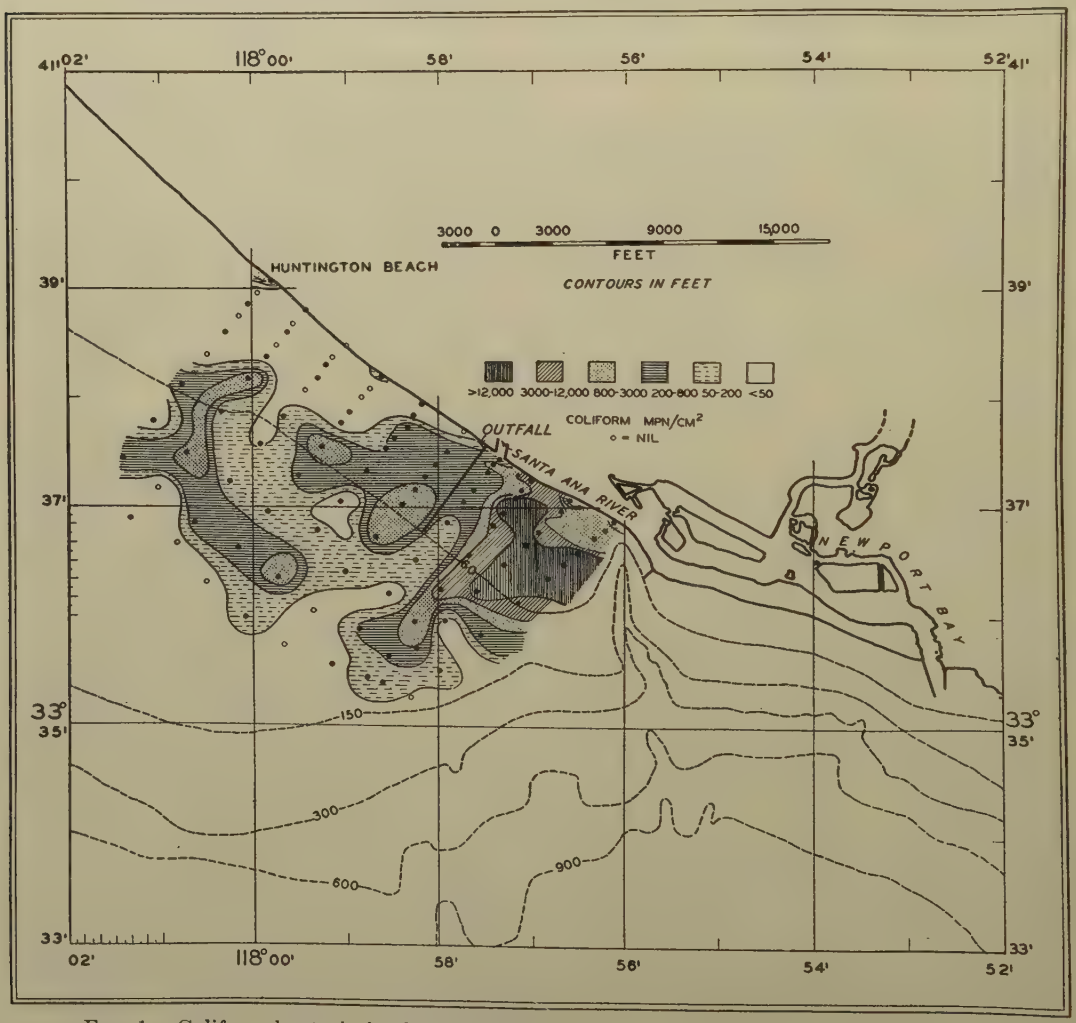

FIG. 1. Coliform bacteria in the sediments around the Orange County sewage outfall

of the time. The character of the area of the Hyperion outfall is very similar to that around the Orange County outfall. There is a flat hinterland, sandy beaches, and a gently sloping bottom. The sediments, for the most part, consist of medium to fine clean sands and sandy silts with dark or black colored sediments rich in organic matter adjacent to the outfall.

The Los Angeles County Sanitation District outfalls are located at White's Point at the foot of the Palos Verdes Hills (Fig. 3). The longest of these outfalls extends about 8000 feet to sea and terminates in 200 feet of water. A total of about 165 million gallons of unchlorinated primary treatment effluent are discharged daily from the White's Point outfalls. The shoreline in the vicinity is steep with rock or gravel beaches, and the sea bottom slopes off rapidly. The bottom sediments range from rock and coarse gravel to fine sands and sandy silts as one proceeds seaward. Free hydrogen sulfide is present in the surface

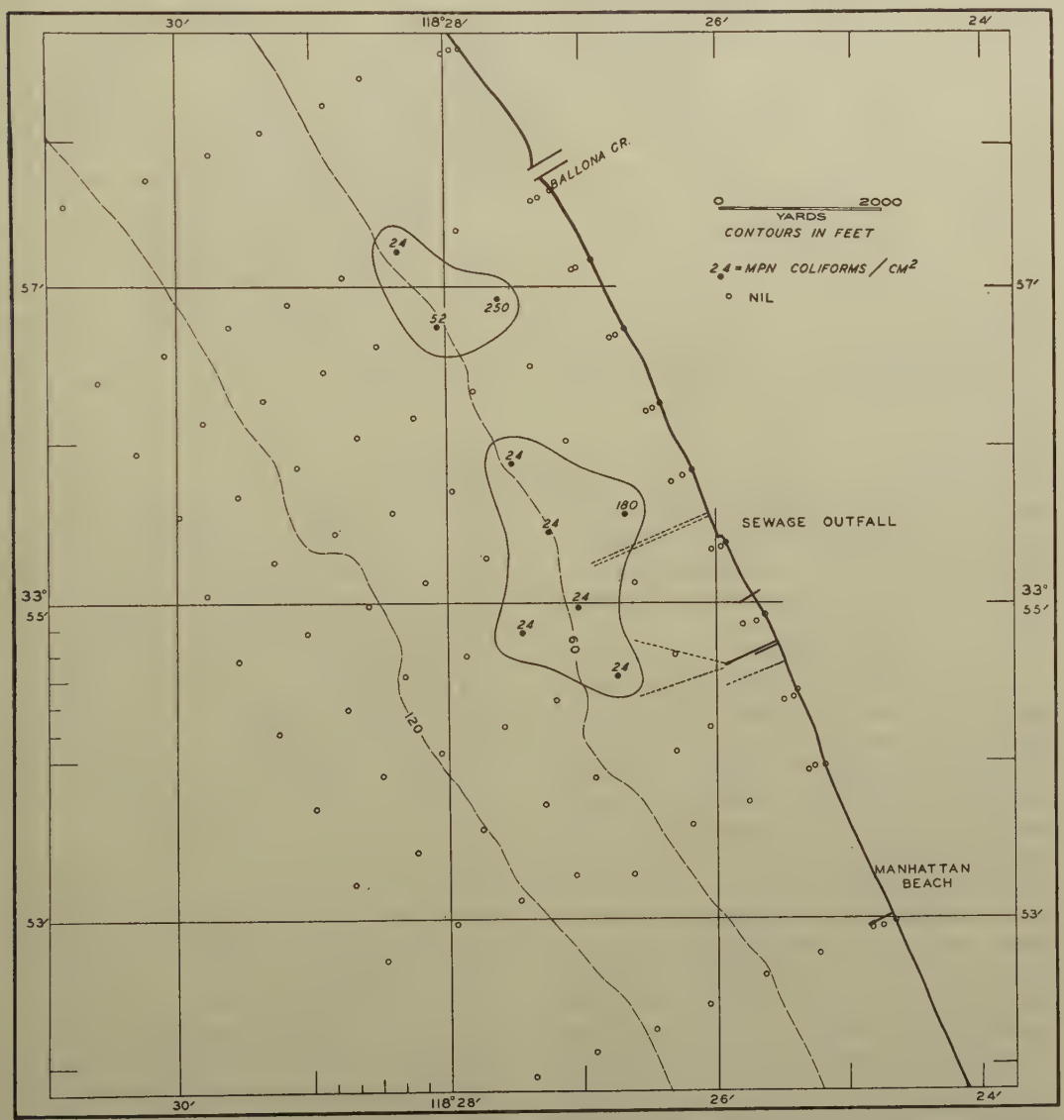

FIG. 2. Coliform bacteria in the sediments around the Los Angeles City (Hyperion) sewage outfall

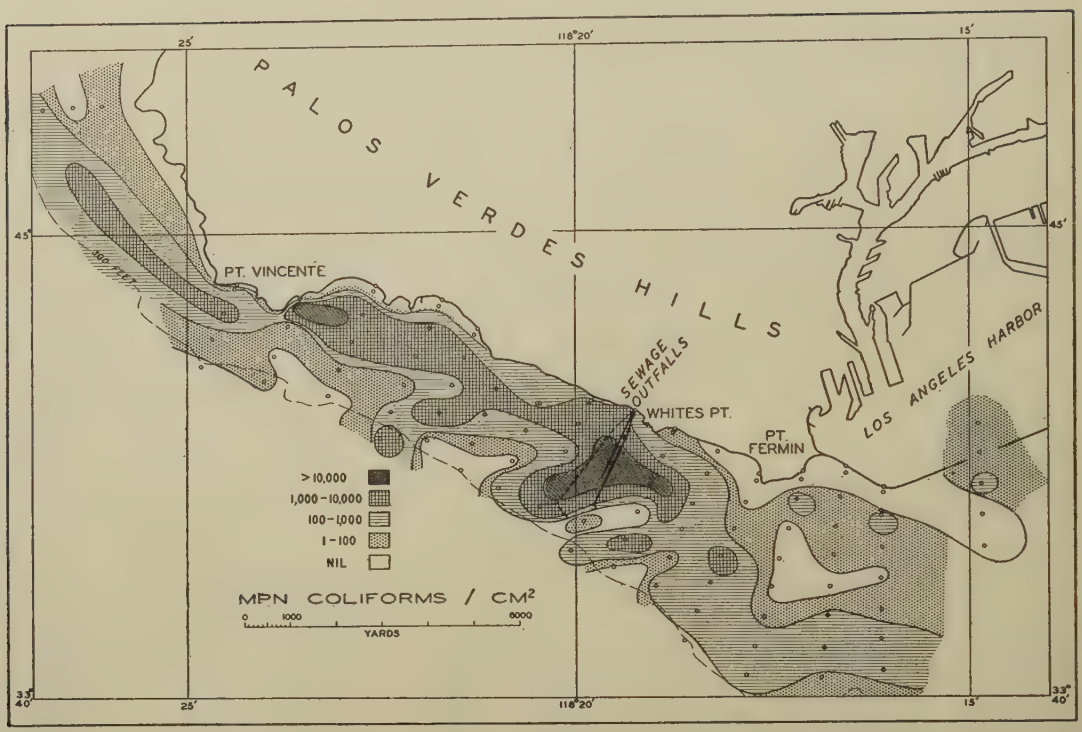

FIG. 3. Coliform bacteria in the sediments around the Los Angeles County (White's Point) sewage outfalls.

layers of the sediments within a radius of roughly one-half to one mile around the outfalls. Sediments having a black color (FeS) extend more than five miles up and down coast from the outfalls and contain a relatively high percentage of organic matter.

METHODS

It was found that the usual methods of collecting sediment samples by means of corers or snappers were inadequate for our purposes. Using either type of device, considerable distortion and disruption of the surface of the sediment occurs either during the collection of the sediment or during its removal from the sampling device. Samples taken for analysis consequently contain a highly variable amount of the surface material. Since the viable coliform bacteria, introduced by settling, should be present largely or exclusively in a thin surface layer, the coliform counts obtained with these methods of sampling are more likely to reflect the proportion of the surface material present in the sample removed for analysis than the actual abundance of coliforms in the area.

For the present investigation, samples were collected by two methods designed to obviate this difficulty. Divers were employed to collect the sediments in shallow water and in the surf zone, where the ship could not operate. The divers were provided with glass tubes open on both ends, about 1.8 cm in diameter and 6 cm long, having a cross sectional area of 2.5 cm^2. The tubes were sterilized, filled with sterile tap water, and closed with sterile rubber caps that fitted over the ends. To take a sample, the diver removed the caps while holding the tube just above the sediment surface, pushed the tube into the sediment and replaced the caps without allowing any of the sediment to escape. Although a somewhat variable depth of the sediment column is sampled by this procedure, the entire surface layer is captured, and this layer is the significant portion of the sample.

When the tubes were returned to the divers' launch, the entire contents were

transferred to sterile water blanks of proper volume to give an initial dilution of 2.5 cm^2 of surface area per 100 ml of suspension. Appropriate further dilutions were prepared and coliform populations determined. The results obtained are reported as most probable numbers (MPN) per cm^2 of sediment surface.

For shipboard work, a sampling device designed by Emery (1958) was employed. This device also samples a 2.5 cm^2 area, and as with the divers' samples, the entire surface layer is captured. At several stations samples collected by both techniques gave comparable coliform counts.

The samples were analyzed for coliforms following Standard Methods for Analysis of Water, Sewage and Industrial Wastes (American Public Health Association 1955) in all respects. Dilutions were prepared and presumptive tubes inoculated within an hour after the sediments were obtained, so that problems introduced by the storage of samples were eliminated. All positive presumptive tubes were confirmed on eosin methylene blue (EMB) agar; brilliant green bile broth was unsuitable for the confirmed medium for reasons mentioned later. All doubtful confirmed tests were completed. No effort was made to distinguish critically between the various species of coliforms present. However, a number of pure cultures were isolated at random from typical colonies appearing on the EMB plates, and these cultures were examined by the usual morphological and biochemical (imvic) tests employed for the identification of coliforms (American Public Health Association 1955). In all cases, cultures picked from typical colonies proved to be coliforms.

RESULTS

One-hundred samples were collected in the vicinity of the Orange County outfall on a series of parallel lines perpendicular to the shore from about 1.5 miles down coast to 3 miles up coast from the outfall. On each line, one sample was taken at the water line on the beach, one in the surf, and the remaining 8 were evenly spaced out to about 3 miles offshore. Using similar grid patterns, 106 samples were collected around the White's Point outfalls, and 109 samples were taken in Santa Monica Bay around the Hyperion outfall.

TABLE 1. *Coliform bacteria in the sediments around three marine sewage outfalls*

Location	Number of samples	Number positive	Range of MPN coliforms/cm^2
Orange County	100	83	nil to 25,000
White's Point	106	72	nil to 92,000
Santa Monica Bay	109	9	nil to 250

Table 1 shows the number of positive samples and the range of MPN's observed at each location. Figures 1, 2, and 3 map the extent and intensity of the bottom coliform fields found in the three areas.

It can be seen that the sediments in an extensive area around the Orange County (Fig. 1) and White's Point (Fig. 3) outfalls, where unchlorinated effluent is constantly being discharged, contain large numbers of coliform bacteria. In the vicinity of the Orange County outfall (Fig. 1), the highest counts were observed in sediments down coast from the outfall extending shoreward into the beach. One sample, taken at the water line about 3000 feet down coast from the outfall position, had a MPN of 5,200 per cm^2. It should be mentioned that this sample, as well as other high count samples, appeared to consist of clean sand with no visible evidence of sludge deposits.

The distribution of coliforms in the sediments around White's Point (Fig. 3) differed somewhat from that at Orange County although an equally large, if not more extensive, field existed. The highest concentrations of coliforms were found up coast from the outfall in an area parallel to the shoreline extending beyond Point Vincente about 5 miles away, and down coast in an area extending seaward past Point Fermin. The data also show coliforms at the entrance of Los Angeles Harbor which could have come from an outfall discharging inside the breakwater. Contrary to what was found around the Orange County outfall, the water line samples with the exception of the two stations directly inshore of the outfall were negative.

Field observations showed that some of

the sediment samples taken in the White's Point area were black in color (FeS) and some smelled strongly of hydrogen sulfide. The occurrence of sulfides in the surface layers of these sediments where the bottom topography does not restrict water movement (and hence availability of oxygen) can only be explained by an abnormally high organic matter content which allows bacterial activity to establish anaerobic conditions (Emery and Rittenberg 1952). In addition, grab samples taken with a clam shell snapper sometimes contained large masses of tubes of the marine worm *Chaetopterus variopedatus*, another indicator of high organic matter content, which in this area could be only of outfall origin. It can be seen from Figure 4 that the distribution of these markers of pollution correlates with the observed distribution of coliforms in the area.

In contrast to what was found in the other two locations, only a few of the sediment samples collected around the Hyperion outfall (Fig. 2) showed the presence of coliforms, and in these samples the numbers were low. In an earlier survey, in which samples were taken with a corer or snapper, 29 positives were obtained out of 101 samples collected over essentially the same area. Since the spacing of the samples was different in the two surveys, the difference in the percentage of positives is of little significance. The less extensive coliform field, both with respect to area and numbers, is perhaps what one would expect considering that essentially all the effluent discharged into this area is chlorinated and low in coliforms.

Although the samples from the area of the Hyperion outfall contained few or no coliforms, they almost always gave positive presumptive tests when 1 ml of the lowest dilution (2.5 cm^2/100 ml) was used as the inoculum. The large majority of these positive lactose broth tubes failed to confirm on EMB agar, and an effort was made to determine the cause of these false positive presumptives. It was found that at least part of these false positives were due to

FIG. 4. Distribution of black sediments, hydrogen sulfide, and *Chaetopterus variopedatus* in the vicinity of the Los Angeles County (White's Point) sewage outfall.

lactose fermenting anaerobic spore-forming bacteria. More important from the point of view of methodology, it was found that about 50 per cent of the positive lactose tubes that failed to confirm on EMB agar gave positive results in brilliant green bile broth despite the absence of coliform bacteria in the samples. These data show that brilliant green bile broth should not be used for the confirmed test in any project dealing with marine sediments.

DISCUSSION

The finding of extensive fields of coliforms in the sediments around outfalls discharging sewage effluent into the oceans raises the question as to the mechanism responsible for their observed distribution. Studies have been made of the current systems and the movements of the surface effluent fields in the vicinity of the three outfalls using a variety of techniques: current meter measurements, observations of drogues, drift cards and dye patches, calculations from salinity determinations and aerial photography (Stevenson *et al.* 1956, Rittenberg 1956). The data show that the coliforms in the sediments (and the other markers of pollution in the case of the White's Point area) are, in general, present in the areas beneath the surface movement of the effluent field in the water. It has also been shown, by following the distribution of effluent tagged with radioactive scandium at the outfall inlet on land, that essentially all the effluent discharged from an outfall rises to the surface of the ocean (Rittenberg 1956). Consequently, most of the coliforms must reach the sediment by deposition from above. Other mechanisms, such as transport along the bottom by turbidity currents and scavenger organisms, while possible, are probably secondary.

The occurrence and areal distribution of the coliform fields in the sediments attest to the significance of sedimentation as a major factor in the disappearance of coliforms discharged into ocean waters. The importance of sedimentation in the self-purification of fresh water has long been known (Jordan 1900, Gainey and Lord 1952), and this process has been claimed to have varying degrees of significance in the self-purification of the salt water limans in the vicinity of Odessa (Rubentschik *et al.* 1936), of tidal estuaries (Weiss 1951, Ketchum *et al.* 1952), and of sea water (Dienert and Guillerd 1939). None of the above authors attempted a direct demonstration of coliforms in the sediments of the environments involved. Rubentschick *et al.* (1936) and Weiss (1951) explained the sedimentation effect in terms of the marked adsorption of coliforms on silts, which they demonstrated by laboratory experiments. The latter author also showed that sea water markedly reduced the adsorption of coliforms on river and estuarine silts and could cause desorption of bacteria. However, in the vicinity of the marine sewage outfalls studied, it is probable that flocculation and settling of the sludge released in the effluent is more important in promoting sedimentation of coliforms than is adsorption to the suspended inorganic materials naturally present in these areas (Stevenson 1956).

A much more difficult question to resolve is how long the coliform bacteria survive in the marine sediments. Studies have been made on the distribution of coliforms in the surface and subsurface waters around the same three outfalls by ourselves (Rittenberg 1956) and by personnel of the three sanitation districts involved. These studies show that coliforms can be carried in the water for sufficient distances away from the outfalls to account for their observed distribution in the sediments. However, the numbers in a column of water at any one time are less than the numbers in the sediments below except within perhaps a few hundred feet of the outfall boil. For example, in numerous sampling series in the Orange County outfall area, MPN's of coliforms greater than 10 per ml were never observed in water more than 6000 feet from the outfall boil (excepting the surf zone). In contrast, sediments as far as 3 miles from the outfall have coliform populations measured in thousands or even tens of thousands per cm^2. Thus the coliforms must survive for a sufficiently long period of time in the sediments to allow large populations to be

built up by deposition from the overlying low count water, although no precise estimate can be made of the survival period. This assumes that there is no multiplication of the coliforms in the sediments, and although this assumption is consistent with prevailing opinion about the behavior of coliforms in sea water (see reviews previously cited), it has not to the best of our knowledge been put to experimental test with respect to marine sediments.

Whatever the survival time may be, the steady state condition is such that significant populations do exist in the surface layer at any one time. Since the coliform field in the sediments can extend to the water line on the beach, the question arises as to whether or not the existence of the field has public health significance. Since the coliforms are present there is no known reason why enteric pathogens could not be present in the same environment. If they are present in the sediments of the surf zone where wave action could resuspend them in the water, it is not outside the realm of possibility that a public health hazard exists for those individuals using the area for recreational purposes. It must be emphasized that there is no epidemiological evidence that infection has resulted from such a situation. It is suggested that a more detailed investigation of this problem is in order.

REFERENCES

American Public Health Association. 1955. Standard methods for the examination of water, sewage, and industrial wastes. A. P. H. A., New York. 10th ed. 522 pp.

Dienert, F., and A. Guillerd. 1939. Studies on the pollution of sea water by the discharge of sewage. Bull. Men. Off. Int. Hyg., Publ. **31**: 869.

Emery, K. O. 1958. Bacterial bottom sampler. Limnol. & Oceanog., **3**(1): 109–111.

Emery, K. O., and S. C. Rittenberg. 1952. Early diagenesis of California basin sediments in relation to origin of oil. Bull. Amer. Assoc. Pet. Geol., **35**: 735–806.

Gainey, P. L., and T. H. Lord. 1952. Microbiology of water and sewage. Prentice-Hall, New York. 430 pp.

Greenberg, A. E. 1956. Survival of enteric organisms in sea water. U. S. P. H. S., Pub. Health Repts., **71**: 77–86.

Jordan, E. O. 1900. Some observations upon the self-purification of streams. J. Exp. Med., **5**: 271–314.

Ketchum, B. H., J. C. Ayers, and R. F. Vaccaro. 1952. Processes contributing to the decrease of coliform bacteria in a tidal estuary. Ecology, **33**: 247–258.

Nusbaum, I., and R. M. Garver. 1955. Survival of coliform organisms in Pacific ocean coastal waters. Sew. Indust. Wastes, **27**: 1383–90.

Pearson, E. A. 1955. An investigation of the efficiency of submarine outfall disposal of sewage and sludge. State Water Pollution Control Board, Std. Serv. Agreement No. 12C-14, State of Calif. 258 pp.

Rittenberg, S. C. 1956. Studies on coliform bacteria discharged from the Hyperion outfall. Final report to the Hyperion Engineers, Inc., Univ. of Southern Calif. 51 pp.

Rubentschik, L., N. B. Roisin, and F. M. Bieljansky. 1936. Adsorption of bacteria in salt lakes. J. Bacteriol., **32**: 11–31.

Stevenson, R. E. 1956. Unpublished data.

Stevenson, R. E., R. B. Tibby, and D. S. Gorsline. 1956. The oceanography of Santa Monica bay, California, I and II. Final report to the Hyperion Engineers, Inc., Univ. of Southern Calif. 407 pp.

Terry, R. D. 1956. An annotated bibliography on bacteriology, oceanography and marine geology as they relate to the disposal of waste material into the sea. Allan Hancock Foundation, Univ. of Southern Calif. 112 pp.

Weiss, C. M. 1951. Adsorption of *E. coli* on river and estuarine silts. Sew. Indust. Wastes, **23**: 227–237.

ZoBell, C. E. 1946. Marine microbiology. Chronica Botanica, Waltham, Mass. 240 pp.

Bacterial Bottom Sampler[1]

Preliminary studies of pollution near sewage outfalls of southern California showed the presence of a well-established field of coliform bacteria in a thin surface layer of marine bottom sediment. The early samples were cut from the tops of cores or from snapper samples. Because most of the sediments were sandy, cores usually were difficult to obtain, most of them slipping out of the core barrel while being hoisted out of the water; in addition, much of the surface layer was lost by dragging against the inside of the core barrel. Samples taken by the snapper had obviously been disturbed and mixed when the jaws of the instrument closed. Both devices permitted possible contamination during lowering and hoisting through the overlying waters that were known to contain coliforms recently discharged from the sewage outfalls.

For ordinary bacteriological studies of marine sediments little contamination from the overlying water is possible, and so most studies have been based on samples obtained by snappers, corers, or other standard devices. A suction device developed by Renn (ZoBell 1946, pp. 33–36; APHA 1955, pp. 460–467) opens only at the bottom, but since suction may draw in sediments from the surrounding area, there is uncertainty about the precise area sampled.

It became evident that a new type of sampler was required, one which could (1) collect an undisturbed sample of the thin top layer of sediment, (2) not be contaminated by the overlying water, and (3) be operated at any depth of water (especially depths too great for divers to work). These specifications were translated to mean that the instrument should contain a sterile receptacle that would be closed during descent, would open to receive a sample of sediment at the bottom, would close again during hoisting, and could be operated at the end of a long light-weight cable.

On the basis of a rough sketch, Mr. Alexander Campbell, Chief Engineer of the University of Southern California's research ship, *Velero IV*, built the device illustrated in Figure 1. The general structure is of brass, weights attached to the foot are of lead, and the sample receiver is a 4-dram short-style shell vial (21 mm outside diameter, 70 mm length). Total weight is 9 kg. In operation, a sterilized vial is filled with sterile sea water, inserted into the brass holding tube, and the device is cocked so that the end of the vial is covered by the cut-off plate. During the lowering to the bottom, water can neither enter nor leave the vial. On contact with the bottom, the flat foot rests atop the bottom while the main housing continues to drop. The relative closure of the foot and housing activates the simple mechanism illustrated in Figure 1 so that the cut-off plate is pushed aside and the glass vial penetrates into the bottom about 5 mm. Continued closure of housing and foot trips the cut-off plate so that it snaps back into its original position, in the process cutting off the sediment that had entered the vial. Hoisted off bottom, the housing and foot move apart, locking the cut-off plate in position so that the sediment within the vial cannot escape. On recovery aboard ship the vial is removed and an aliquot portion of the sample removed for making bacterial dilutions.

After being properly adjusted, the device in its final form was able to collect a sample about nine times out of ten lowerings. Some misses occurred when it hit rock bottom, some in very soft mud, and some possibly because rapid ship drift caused it to strike on edge instead of flat on the foot. A small amount (less than ten per cent) of the sediment that entered the vial was washed out by water from within the vial that had to escape to make room for the sediment.

[1] Contribution of Allan Hancock Foundation No. 205.

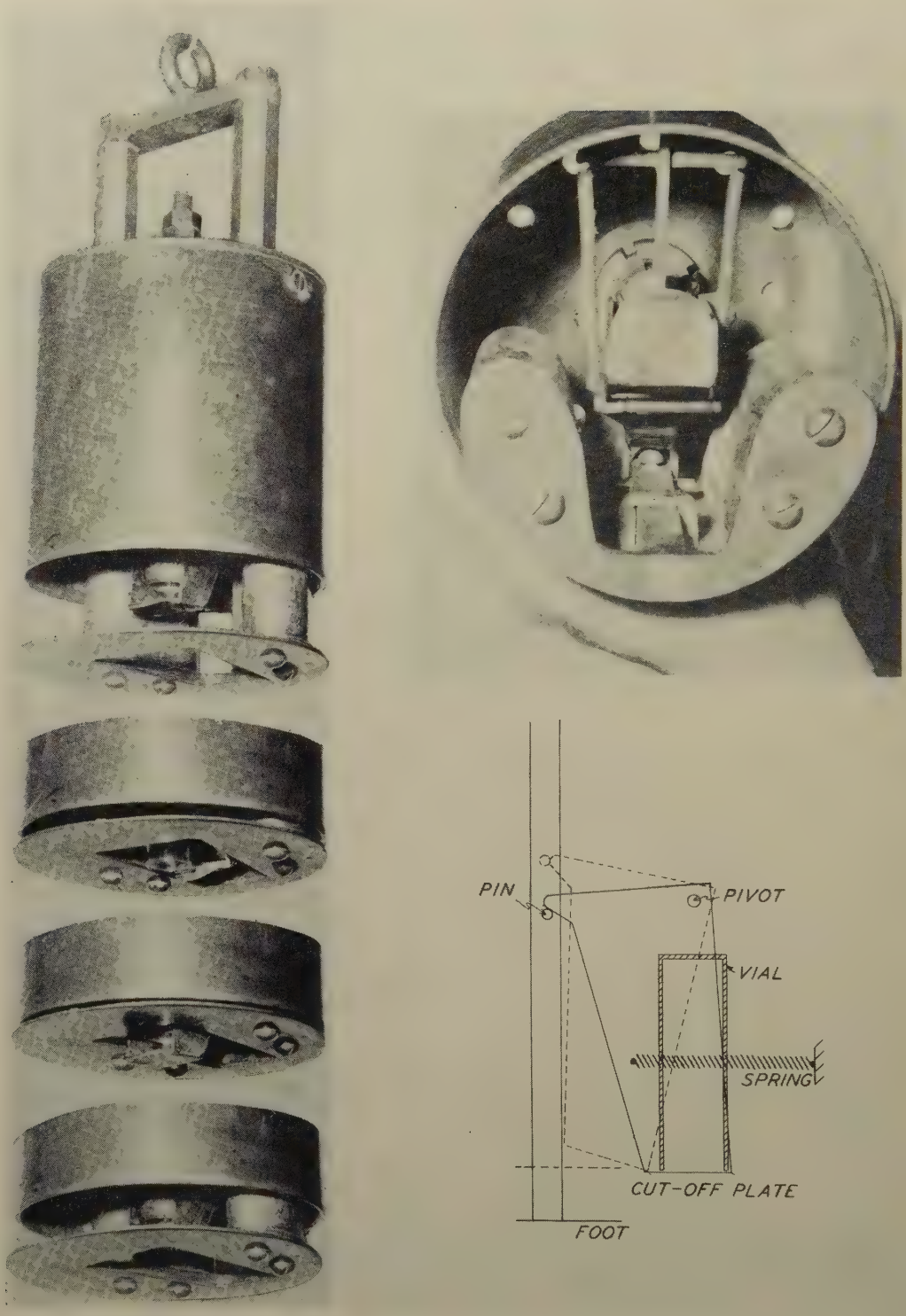

FIG. 1. Bacterial Bottom Sampler. *Left top*—sampler cocked and ready for lowering. *Left second*—contact with bottom drives foot upward and causes the cut-off plate to be withdrawn so that the glass vial penetrates the sediment. *Left third*—at rest on bottom, the foot closes against the housing, releasing the cut-off plate so that it snaps off the sediment column at the end of the glass vial. *Left bottom*—sampler being hoisted back to the surface in the tripped and locked position. *Right top*—view into bottom of sampler with foot plate removed. *Right bottom*—diagram of essential working parts of sampler.

Unlike most other samplers, this one worked better on sand than on mud bottom. Three separate surveys described by Rittenberg, Mittwer, and Ivler (1958) were based on samples obtained with this device plus others taken by divers in depths too shallow to be reached by the ship. It is believed that the bacterial bottom sampler may also be useful for other specialized work, such as population studies of living Foraminifera; however, for some such purpose a larger version might be preferable.

REFERENCES

American Public Health Association, Inc. 1955. Standard methods for the examination of water, sewage, and industrial wastes. New York. 522 pp.

Rittenberg, S. C., T. Mittwer, and D. Ivler. 1958. Coliform bacteria in sediments around three marine sewage outfalls. Limnol. & Oceanogr., 3(1): 101–108.

ZoBell, C. E. 1946. Marine microbiology. Chronica Botanica, Waltham, Mass. 240 pp.

K. O. Emery

Department of Geology, University of Southern California, Los Angeles, California

The Mucilage Tubes of *Amphipleura rutilans*[1]

INTRODUCTION

Amphipleura rutilans (Trentepohl) Cleve [= *Berkleya obtusa* (Grev.) van Heurck] is a common littoral diatom of the Atlantic coast. The cells characteristically grow colonially in branched tubes, which may form dense tufts 2–5 cm in height. Such pseudothalli are commonly to be found in spring, in pools or on intertidal rocks about mid-tide level (Aleem 1949), where they may readily be mistaken for thalli of filamentous Phaeophyceae.

Küster (1937) found *Amphipleura rutilans* at all seasons in waters of a brine spring at Bad Nauheim which contained 4.5% NaCl. He obtained uni-algal isolates, and grew these on 2% agar media, in which mucilaginous tubes 1–2 cm long were formed in 2–3 months. Their staining reactions in gentian violet or ruthenium red indicated that these tubes might be composed of pectic material. The mucilage tubes of several other colonial diatoms likewise showed metachromasy in basic dyes such as methylene blue (von Cholnoky 1927). The capsules of *Navicula pelliculosa*, which reacted similarly, were shown to be composed of an acidic polysaccharide built up of glucuronic acid units (Lewin 1955). This appears to be the only analysis of a diatom mucilage which has been published hitherto.

[1] Issued as N.R.C. No. 4574.

EXPERIMENTAL METHODS AND RESULTS

a. Separation of diatom colonies

Pseudothalli of *Amphipleura rutilans* were collected at Herring Cove, N. S., on 25 March 1955. (The species was kindly identified by Dr. P. Conger, Smithsonian Institution, Washington, D. C.). Since it was not possible in the field to distinguish the tufts from those of *Ectocarpus*, they were separated in the laboratory on the basis of the marked metachromasy of the diatom mucilage. The mixture of algae was rinsed in tap water and soaked overnight in 95% ethanol. Most of the chloroplast pigments leached out, and the rest were removed by further rinsing in ethanol. The fibrous mass was then soaked in water, in which it rapidly rehydrated, and toluidine blue was added. The diatom colonies stained a lavender pink, while those of *Ectocarpus* took on a greenish-blue color. The former were picked out, freed from foreign material, decolorized in ethanol slightly acidified with HCl, washed, and dried in a forced draft at room temperatures. The dry mass was powdered in a mortar and analyzed as described below.

b. Isolation of mucilage tube material

From about 100 g (squeezed wet weight), 1.2 g of diatom pseudothalli free from gross contamination was obtained. After extrac-

tion with 50 ml 0.5% KOH at 120° for 15 min, the cell residue (R.1) was separated by centrifugation. The supernatant extract (S.1), together with rinsings from R.1, was acidified to pH 2.0 with HCl, and a small precipitate was removed (9 mg, largely protein). The solution was then poured into 200 ml 95% ethanol, resulting in a voluminous, stringy precipitate, which was rinsed with 95% ethanol, dried and powdered; yield 587 mg (R.2), *i.e.*, approx. 50% of the original dry weight. The supernatant ethanol solution (S.2) was evaporated to dryness, leaving a negligible residue composed chiefly of KCl.

The undissolved cell fraction (R.1) was subjected to a second extraction with 20 ml 0.5% KOH at 120° for 30 min. The supernatant extract (S.3) was acidified as before, and a precipitate (R.4) was removed which proved to consist of 43 mg of crude protein with only traces of polysaccharide. The supernatant solution (S.4) was poured into 3 volumes of alcohol; no precipitate formed, indicating that virtually all the mucilaginous material had been removed from the cells on the first extraction (S.1).

c. Cell residues

The cell residue (R.3) appeared to consist almost entirely of diatom frustules. It was rinsed and dried, yielding 265 mg, in which ash and silica were determined gravimetrically as follows. A 20-mg sample (W.1) was burned at red heat in a platinum crucible, cooled, and weighed (W.2). It was then heated with concentrated H_2SO_4 to convert oxides, chlorides, etc., into sulphates, and weighed again (W.3). Finally, it was heated with a mixture of HF and H_2SO_4 until no more SiF_4 was evolved, raised to red heat to drive off excess acids, cooled, and weighed once more (W.4). The ash content (W.2/W.1) was found to be 73%, of which 93% $\left(\frac{\text{W.3} - \text{W.4}}{\text{W.2}}\right)$ was SiO_2; hence the weight of silica frustules was about 180 mg, or 15% of the total dry weight of the diatom colonies.

d. Analysis of tube material

1) *Carbohydrate.* Samples of the precipitated material (R.2) were hydrolysed in 0.7% H_2SO_4 at 100° for 6 hr, and after neutralization with $BaCO_3$ were examined by paper chromatography (Lewin 1956). Comparisons with known reference sugars indicated predominantly xylose and mannose, with traces of rhamnose and two unidentified components with low R_f values. No hexosamine could be detected in hydrolysates by the reaction of Morgan and Elson (Partridge 1948).

2) *Protein.* By micro-Kjeldahl determinations, the material was found to contain 4.6% organic nitrogen, evidence for as much as 30% protein. Two-dimensional chromatograms of more completely hydrolysed samples revealed a dozen ninhydrin-positive components, indicating amino-acids of the usual variety found in proteins.

3) *Sulphate.* The crude tube material (R.2) also contained 10% non-volatile ash. Since the acidic polysaccharides of marine algae frequently contain ester sulphate groups which combine with metallic cations, gravimetric determinations of sulphate were carried out on the mucilaginous material (R.2) as follows. A 20-mg sample was digested in a sealed tube with 1.0 ml concentrated HCl plus 0.05 ml HNO_3 at 100° for 24 hours. It was then rinsed out with 0.5 ml water, and traces of solid residue removed by centrifugation. To the supernatant, including rinsings, 1.0 ml of 2% $BaCl_2$ was added, and the mixture was boiled to coagulate the $BaSO_4$. The precipitate was then rinsed by centrifugation, transferred to a tared watch-glass, dried overnight at 100°, and weighed. Of this weight, 41% was SO_4, from which the sulphate content of the original sample (R.2) was calculated. An average of two determinations indicated 16% sulphate. This

value exceeds that of 10% obtained for the ash content of the mucilage tube material (R.2), since the ash comprises only non-volatile salts, free sulphuric acid having been driven off by incineration.

4) *Silica.* Finally, since the method of extraction of the tube material involved digestion in alkaline solution, the reprecipitated material (R.2) was analyzed for traces of silica. This was determined colorimetrically, after fusion of a sample with excess Na_2CO_3 and extraction with water, by measuring the intensity of the color of reduced silico-molybdate (Lewin 1954). Only about one-tenth of the ash could be accounted for as silica.

DISCUSSION

Though the material isolated from the diatom colonies contained both polysaccharide and protein, it remains to be established whether these components were originally associated, in the form of muco-protein, in the tubule walls, or whether the protein had been leached out of the cells and co-precipitated with the polysaccharide under the preparative technique employed. The purpose of this note is to indicate that such tubules do not consist of pectin, as has been supposed on the basis of their staining reactions. No further work on their chemical composition is contemplated by the present author.

REFERENCES

ALEEM, A. A. 1949. Distribution and ecology of marine littoral diatoms. Bot. Notiser, **4**: 414–440.

KÜSTER, E. 1937. Die Gallertbildungen der *Amphipleura rutilans*. Arch. Protistenk., **88**: 211–235.

LEWIN, J. C. 1954. Silicon metabolism in diatoms. I. J. Gen Physiol., **37**: 589–599.

———. 1955. The capsule of the diatom *Navicula pelliculosa*. J. Gen. Microbiol., **13**: 162–169.

LEWIN, R. A. 1956. Extracellular polysaccharides of green algae. Can. J. Microbiol., **2**: 665–672.

PARTRIDGE, S. M. 1948. Filter-paper partition chromatography of sugars. Biochem. J., **42**: 238–251.

VON CHOLNOKY, B. 1927. Beiträge zur Kenntnis der Bacillariaceen-Kolonien. Hedwigia, **67**: 223–226.

RALPH A. LEWIN

Atlantic Regional Laboratory, National Research Council, Halifax, N. S., Canada[2]

[2] Present address: Marine Biological Laboratory, Woods Hole, Massachusetts.

Proceedings of the American Society of Limnology, and Oceanography, Inc.

Minutes of the 20th Annual Meeting
Stanford, California
August 28, 1957

The 20th Annual Meeting was called to order at 11:05 A. M. by President Chandler.

The Minutes of the 19th Annual Meeting were approved as printed in Volume II, No. 1 of LIMNOLOGY AND OCEANOGRAPHY.

President Chandler welcomed the new members of the Society and expressed his appreciation to those members present for the stimulating papers delivered at the 20th Annual Meeting.

The President announced that the 21st Annual Meeting would be held in Bloomington, Indiana, in August 1958, and that the 22nd Annual Meeting will be held in August 1959 at Pennsylvania State University. Both of these meetings are to be held in conjunction with the meetings of the American Institute of Biological Sciences.

The President discussed the progress in the plans to invite the International Limnological Association to hold its meeting in 1962 in the United States. He announced that Dr. Arthur D. Hasler had been appointed Chairman of the Special Committee with Dr. Bostwick H. Ketchum as Ex Officio member. Dr. Hasler had informed the President that it would be possible to hold this meeting at the campus of the University of Wisconsin, and the President announced that the Board of Directors had authorized Dr. Hasler to select his committee members, to proceed with the organization of such a meeting, and to extend the invitation to the International Limnological Association.

The President announced the appointment of the following members to committees of the Society to serve through the indicated year:

Apparatus and Supplies
George H. Lauff, 1959

Nominating Committee
Claude E. ZoBell, Chairman, 1957
Robert C. Ball, 1957
Dean F. Bumpus, 1957

Interim Advertising Committee
Frank F. Hooper, Chairman, 1957
John H. Ryther, 1957

Interim Library Subscription Committee
F. C. W. Olson, 1957

Auditing Committee
Herbert W. Graham, 1957
Arthur R. Miller, 1957

Local Chairman for ASLO 20th Meeting
Donald E. Wohlschlag, 1957

Identification Service
John L. Brooks, 1959

The Secretary reported that the total membership of the Society is 751, of whom 18 are student members, six are family members, and 12 are emeritus members. The remaining 715 members are full members of the Society and are subscribing to LIMNOLOGY AND OCEANOGRAPHY. During the last year 72 active members and two associates have joined the

Society, 8 members have resigned, and 110 members have been dropped for non-payment of dues. None of these 110 members who have been dropped for non-payment of dues had subscribed to Volume I of LIMNOLOGY AND OCEANOGRAPHY. 41 members who subscribed to Volume I have not yet subscribed to Volume II of the journal.

The Secretary has recorded with regret the deaths of Lawrence E. Hicks and George D. Louderback.

Library subscriptions to LIMNOLOGY AND OCEANOGRAPHY have increased to 147. Most of the libraries and new members have subscribed retroactively to Volume I of the journal so that we are now selling a total of 863 copies of the journal. It was announced that the Board of Directors has authorized the Secretary-Treasurer to investigate the cost of printing a larger number of copies of the journal.

The Secretary reported that the membership list is now grossly out of date. Since it seems that our membership has now stabilized following the large changes coincident with the recent initiation of the journal and increased dues, it was proposed that the Society should consider the desirability of issuing a new membership list. The discussion that followed indicated that some of the members would like to have a list available which was more comprehensive than our membership list alone. It was the concensus of the meeting, however, that our membership list should be published first and that an expanded list could be considered at a later date.

It was moved, seconded, and passed that the Secretary should proceed with the preparation and publication of the new membership list, the contents to be comparable to the membership lists which have been published in the past.

The Treasurer's Interim Report covering the period January 1, 1957 to July 31, 1957 was presented and approved. This report showed a balance on hand of $6,585.43, which includes most of the income for the year but half of the expenses for Volume II of LIMNOLOGY AND OCEANOGRAPHY. The Treasurer reported further the receipt of a check from the National Science Foundation for $1,369.97 in payment of the operating deficit for Volume I of the journal.

Tellers Ketchum and Austin reported that 265 votes had been cast in the memberwide mail ballot. The following officers and members of the Editorial Board were declared duly elected:

President: Dale F. Leipper
Vice-President: Robert W. Pennak
Member-at-Large: Frank F. Hooper
Editorial Board (3-year terms):
Howard T. Odum
William E. Ricker
John H. Ryther

Reports of the following committees and representatives were received and approved:
National Research Council, Lionel A. Walford
American Association for the Advancement of Science, Waldo Schmidt
Apparatus and Supplies, John H. Ryther
Advertising, Frank F. Hooper
Identification Service, Joel W. Hedgpeth
American Institute of Biological Sciences, David C. Chandler

At the 19th Annual Meeting the members instructed the Board of Directors to consider full society membership in the American Institute of Biological Sciences. The Secretary informed the Society that the advantages of full membership would include a subscription

for each of our members to the Bulletin, a voting representative on the Board of AIBS, and the privilege of younger members to purchase low-cost group life insurance. The cost would be $1.00 for each active member of the Society. The Secretary further reported that nearly half of our members are not biologists, and that some of these members had expressed the opinion that they did not feel it necessary to join a national biological society. No action was taken concerning a change in our membership status in AIBS.

The Society expressed a vote of thanks to Dr. Wohlschlag, our representative on the local committee for the 20th Annual Meeting, for his arrangements for our meeting at Stanford, California.

It was moved, seconded, and passed unanimously that the Society go on record as recognizing the extensive and important contribution of Dr. Harold Sverdrup and Dr. Carl G. Rossby to the field of Oceanography and Meteorology, and that the Secretary of the Society be instructed to forward a letter of sympathy and regret to the widows.

There being no further business the meeting was adjourned at 12:10 P. M.

At the Annual Meeting of the Pacific Section of the Society which followed, the election of officers of the Pacific Section was announced:

President: Marston C. Sargent
Vice President: Wayne V. Burt
Members-at-Large:
for 2 years, John R. Brett
for 1 year, Michael Waldichuk

Respectfully submitted,
BOSTWICK H. KETCHUM
Secretary-Treasurer

Financial Statement

	Jan. 1, 1956 to Dec. 31, 1956	Jan. 1, 1957 to July 31, 1957
Balance on Hand	$4,263.69[1]	$3,849.06[2,3]
Receipts		
Dues	5,136.50[2]	4,119.05
Library Subscriptions	1,215.90[3]	1,386.63
Special Publications	17.10	2.85
Reprint Sales	977.54	643.54
Contributions	—	182.00
Interest	99.74	156.48
Total Receipts	$11,710.47	$10,339.61
Expenditures		
Postage	159.87	71.99
Misc. Printing	193.65	123.21
Stenographic Services	473.44	291.51
Contributions—AIBS	100.00	100.00
NRC	25.00	25.00
Travel	91.33	—
Addressograph Service	43.29	24.44
Incorporation Expense	12.00	—
Pacific Section	43.91	—
Miscellaneous	18.05	2.95

LIMNOLOGY AND OCEANOGRAPHY

Manufacture	5,133.42	2,796.51
Address and Mail	345.74	126.94
Reprints	1,041.65	179.93
Editorial Board	121.99	—
Miscellaneous	58.07	11.70
Total Expenses	7,861.41	3,754.18
Balance	3,849.06	6,585.43
Balance on Hand		
Savings Account	3,535.07	6,030.47
Checking Account	313.99	554.96
	$3,849.06	$6,585.43

[1] Includes $1,827.00 of 1956 dues paid in 1955.
[2] Includes $1,407.00 of 1957 dues paid in 1956.
[3] Includes $332.90 for subscriptions to Volume II paid in 1956.

B. H. KETCHUM
Secretary-Treasurer

Errata in Vol. 2 of Limnology and Oceanography

p. 160. Line 1 of left-hand column should read "Serial observations of temperature and salinity".

p. 160. Lines 18 and 19 of right-hand column should read "sigma-t".

pp. 185 and 189. Figures 5 and 10 were inadvertently printed upside down.